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VOL. 18, SEC. C. AUGUST, 1940 NUMBER 8

EFFECT OF NUMBERS OF COLONIES PER PLATE ON THE ESTIMATE OF THE BACTERIAL POPULATION IN SOIL[1]

NORMAN JAMES[2] AND MARJORIE L. SUTHERLAND[3]

Abstract

Numbers of colonies of bacteria on plates from two series of 36 plots were found to vary widely on one date of sampling and from day to day. In an attempt to determine the effect of this variation and of introducing a dilution of different strength during the progress of an experiment on the accuracy of estimates, samples were plated in a series of replicate dilutions, with three parallel plates from each. In Experiment 1, four replicate dilutions of one strength were used. The experiment was repeated 120 times and shows that parallel dilutions, prepared by a slightly altered technique, add only very little to the error of the experiment beyond that of the interaction. In Experiment 2, three replicate dilutions of 1 : 300,000; 1 : 400,000, and 1 : 500,000 strengths, respectively, were used. This experiment was repeated 162 times, yielding a final result similar to that from dilutions of one strength. In the third experiment three replicate dilutions of 1 : 150,000; 1 : 250,000, and 1 : 400,000 strengths, respectively, were used. This experiment was repeated 360 times and shows that parallel dilutions, varying to this extent, introduce an appreciable error to the experiment.

When counts from the 1 : 150,000 dilutions were correlated with those from the 1 : 400,000 dilutions of the same samples, it became obvious that the two systems of diluting do not provide estimates that are interchangeable. This proved true also with counts from five other pairs of dilutions, prepared in Experiments 2 and 3. In each case a curve was fitted to the data from the correlation surface, resulting in a sixth degree curve for the 1 : 150,000 and 1 : 400,000 dilutions, third degree curves for four other pairs of dilutions, and a linear relation for one pair.

The results show that one dilution does not provide an estimate that agrees with that from another dilution of different strength, except at one count. A system of making estimates by using two dilutions and correcting the counts to provide interchangeable estimates is suggested.

The data from Experiment 3 were corrected on this basis and analysed. The corrected data yield an F value for dilutions of different strength about the same as that obtained by the use of parallel dilutions of one strength.

Introduction

In a long term investigation designed to study, among other things, the effect of various cultural practices and fertilizer treatments on the numbers of micro-organisms in soil in relation to the development of root rots in cereals, the averages of four counts from each of 36 plots obtained on one day were found to vary as much as threefold at some platings. Furthermore, the counts varied appreciably at different samplings, yielding averages ranging

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Contribution from the Department of Bacteriology and Animal Pathology, the University of Manitoba, Winnipeg, Man., with financial support from the National Research Council of Canada.

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from 10 to 150 colonies per plate in each season. The higher count is lower than the accepted upper limit in certain fields of study. Still, the probability that the number of colonies developing on plates from such a complex population as is found in soil may affect the accuracy of the estimate must be considered. Too many colonies might introduce the factor of crowding, with consequent scarcity of limiting food material, or mutual relations antagonistic or stimulative. On the other hand, too wide separation of colonies might lead to the lack of stimulation among certain types, resulting in failure to grow and too low a count. Errors attributable to such phenomena might alter the final estimate to such an extent as to conceal real effects in the problem under consideration and lead to unsound conclusions. Accordingly, it appeared advisable to consider the error of estimates from counts varying so widely and the effect of introducing a different dilution in the progress of an experiment in order to provide counts per plate falling within a definite range.

Experimental

Before attempting to determine the effect of introducing a dilution of different strength into an experiment, it appeared necessary to improve the technique so as to reduce the error of parallel dilutions of one strength, or at least to determine the variance attributable to parallel dilutions under the conditions imposed by the technique. Recently, the authors (2) showed that the technique, as commonly used, provides significant differences among dilutions raised in parallel series from one first dilution, and improved the system of diluting appreciably by starting with a 1 : 50 first dilution. After a slight change in procedure, which will be considered in a later report, was adopted, a complex experiment was carried out to obtain information on several points simultaneously. Consequently, data are available from 120 fifty-gram portions of soil diluted 1 : 50 in water. Four dilutions from each original 1 : 50 dilution were raised in parallel series to the 1 : 400,000 dilutions used for plating. Each final dilution was plated in three parallel plates. Then the actual counts were corrected to provide estimates per gram of dry soil. One test supplied estimates as follows:

	Millions of colonies per gram of dry soil		
	Plate 1	Plate 2	Plate 3
Final dilution 1	25.38	24.32	23.80
Final dilution 2	21.68	25.38	30.67
Final dilution 3	27.50	27.50	29.61
Final dilution 4	24.32	30.67	28.56

The experiment provides 120 such tests, each with 3 D.F. for parallel dilutions, 2 D.F. for the estimation of the error of systematic order of plating, and 6 D.F. for interaction or a measure of the inconsistencies of the data in each test.

This interaction may be considered the error appropriate for testing the effect of steps in the technique of the plating method.

In this type of experiment one is not concerned with measuring effects in terms of 5% and 1% levels of significance, but rather with obtaining *F* values close to 1.00 when the number of degrees of freedom for error is large. In this case, the *F* value is the variance for dilutions divided by that for interaction. The variance for a main effect includes the variance for interaction and a portion added by the effect under consideration. Thus, as the values approach 1.00 the portion added by the effect tested approaches zero. The pooled data from the 120 tests give the following results:

Source	D.F.	Variance	*F*	5% Pt.
Replicate dilutions	360	20.3919	1.1077	1.22
Systematic order	240			
Interaction	720	18.4090		

Since the experiment involves a large number of D.F. and the *F* value is close to 1.00, this is evidence that parallel dilutions add only very little to the error of the experiment beyond that of the interaction.

The second experiment was similar to the first except that the three dilutions raised in parallel series gave final dilutions of 1 : 300,000, 1 : 400,000, and 1 : 500,000, respectively. After the counts were corrected to provide estimates per gram of dry soil, the data were analysed as in the previous experiment. The pooled results of 162 tests, each made up of three estimates by each of the three systems of diluting, are presented below.

Source	D. F.	Variance	*F*
Replicate dilutions	324	11.6776	1.1065
Systematic order	324		
Interaction	648	10.5536	

This finding is in close agreement with that of the previous experiment and shows rather clearly that changes in dilutions, within the range considered, add very little to the error of the experiment beyond that added by parallel dilutions of one strength.

The third experiment was designed to test the effects of a wider range in dilutions, but otherwise was the same as the second. The final dilutions were 1 : 150,000, 1 : 250,000, and 1 : 400,000 respectively. The samples considered in this study provided estimates ranging from 10 to 20 millions per gram of dry soil. On this basis the three dilutions from the 20 million population should give counts of 133, 80, and 50 colonies per plate, respectively.

The data represent estimates corrected to the number per gram of dry soil on 360 tests with nine plates each.

Source	D.F.	Variance	*F*
Replicate dilutions	720	16.6258	2.2697**
Systematic order	720		
Interaction	1440	7.3414	

In this case there is evidence that the use of three dilutions, varying to such an extent, introduces an error to the experiment appreciably greater than that added by parallel dilutions of one strength. Further, data not included indicate that differences among counts from this range of dilutions vary from day to day. Since the analysis for each day's plating was based on 324 plates, this finding eliminates the possibility of obtaining a satisfactory correction by averaging estimates obtained from the three dilutions.

The actual counts obtained by means of the three systems of diluting varied within a comparatively narrow range during the progress of the experiment. Accordingly, it would be expected that the estimates based on the different systems of diluting would be interchangeable. To determine this, a correlation surface was prepared, using the means of each set of three counts obtained from the 1 : 400,000 dilutions as x and the corresponding means of sets obtained from the 1 : 150,000 dilutions as y. The values of x ranged from 22 to 54 and, for convenience in fitting the curve, were divided into 17 classes with class intervals of two. Those for y ranged from 55 to 130. In this case the class interval was one. The mean of the y values placed in each class of x was calculated. These 17 means were plotted. The theoretical count for each dilution was determined on the basis of a population producing from 10 to 20 million colonies per gram of dry soil. The results made it obvious that the data from the two systems of diluting do not provide estimates that are interchangeable. Counts of 70 obtained from the 1 : 150,000 dilution were approximately 10% too high as compared with the theoretical. Those of 100 from dilutions of the same strength were about 40% too low. In order to determine whether this type of relation among counts obtained from different dilutions was consistent, a similar procedure was followed using 1 : 200,000, 1 : 250,000, 1 : 300,000, and 1 : 500,000 dilutions as y respectively, with the 1 : 400,000 dilution as x; and also counts from the 1 : 300,000 against those from the 1 : 500,000 dilutions. Data for these tests were obtained from Experiments 2 and 3. The results show a similar trend in all cases except those including the 1 : 500,000 dilutions. Contrary to expectation based on the phenomenon of crowding, estimates from the 1 : 500,000 dilutions were slightly lower, rather than higher, than those from the 1 : 400,000 dilutions.

The data do not appear to follow a linear relation. Accordingly, they were coded and in each case a curve was fitted, following the method for fitting

polynomials outlined by Goulden (1). The calculations in the table on page 230 were carried out according to directions given on page 84. Third degree curves were obtained in all except the 1 : 150,000 with the 1 : 400,000 dilutions, in which case a sixth degree curve gives the best fit. The 1 : 500,000 and 1 : 400,000 dilutions give a linear relation. The following note was provided by Dr. Goulden:

"In the calculation of the polynomial equation the actual class values were replaced by the natural numbers beginning with 1. To deduce the actual equation it was necessary, therefore, to decode. If the calculated equation is:

$$Y' \text{ equals } c_0' + c_1'x' + c_2'x'^2 + c_3'x'^3$$

and if i_y and i_x are the actual class intervals of y and x, then the actual equation is:

$$Y \text{ equals } y_0 + i_y\left(c_0' + c_1'\left(\frac{x - x_0}{i_x}\right) + c_2'\left(\frac{x - x_0}{i_x}\right)^2 + \ldots.\right)$$

where y_0 and x_0 are chosen so that

$$\left(\frac{y_1 - y_0}{i_y}\right) \text{ equals } \left(\frac{x_1 - x_0}{i_x}\right) \text{ equals } 1$$

y_1 and x_1 being the first of the actual class values of y and x."

The results are shown in Fig. 1. It may be noted that the curve intercepts the line for the theoretical at about the same point in each case, and that the counts give estimates that are interchangeable only at this point of intersection. Thus, the greater the difference in count from that of the point of intersection, the greater the discrepancy between the actual and theoretical. In each case the curve at the left of the intersection shows a tendency for the counts plotted as y to be too high in comparison with the theoretical. Counts of the same size to the right of the point of intersection are lower than the theoretical. This would appear to be an inconsistency if it were not for the fact that dilutions of the same strength were taken as x in each case. The discrepancy apparently results from the fact that the counts taken as x values become too low when there are too few colonies in the plate.

These results provide convincing evidence that dilutions of one strength fail to give any indication of the errors of estimates produced by crowding of colonies on plates, on the one hand, and too wide separation of colonies on other plates, on the other. Neither do they furnish any clue as to the number of colonies per plate that is least affected by these factors under the conditions of an experiment. Obviously, that number might not be the same under different soil conditions.

Since it is not possible to predict the range in counts likely to be encountered in an extended experiment, and, likewise, since the range from dilutions of one strength may be so wide as to produce serious errors in some of the estimates, there appears to be no alternative other than to suggest the use of two dilutions of different strengths and the application of a correction to adjust estimates based on the counts affected most seriously by this plate factor error. This

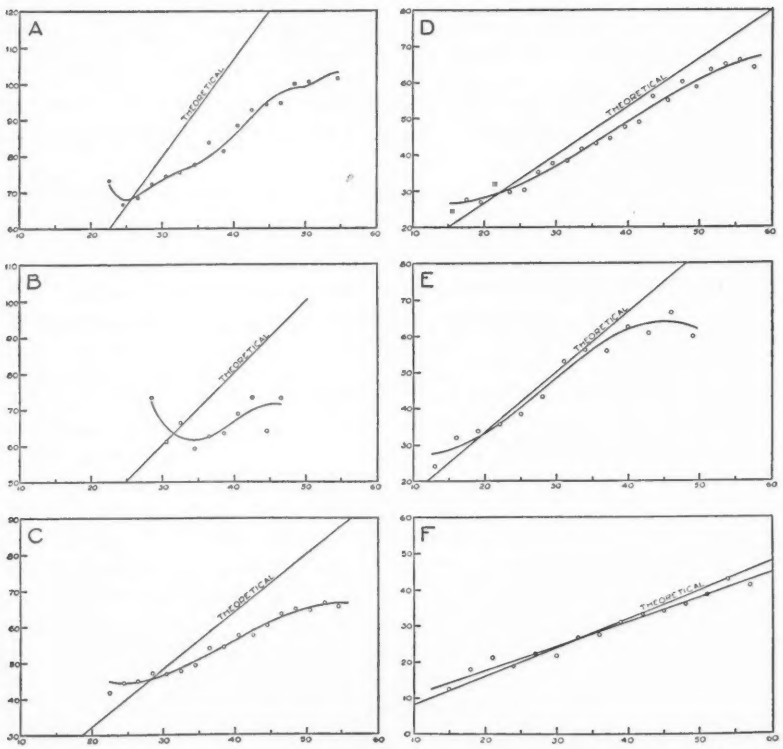

FIG. 1. *Plate counts of soil bacteria from samples plated simultaneously in two dilutions of different strengths. Dots represent means of y values placed in each class of x. Curves fitted according to the polynomial equation. Straight lines represent theoreticals.*

A. 359 pairs of dilutions; y = 1/150,000 and x = 1/400,000
B. 36 pairs of dilutions; y = 1/200,000 and x = 1/400,000
C. 323 pairs of dilutions; y = 1/250,000 and x = 1/400,000
D. 159 pairs of dilutions; y = 1/300,000 and x = 1/400,000
E. 159 pairs of dilutions; y = 1/500,000 and x = 1/400,000
F. 159 pairs of dilutions; y = 1/300,000 and x = 1/500,000.

involves the preparation of a curve similar to the curves in Fig. 1, and from it a simpler correction curve similar to the curves in Fig. 2. The latter represent the values for the curves of Fig. 1 plotted as deviations from the theoreticals based on interchangeability of dilutions. Either curve will show the point of intersection, or the neutral count for each dilution where a correction is unnecessary.

For simplicity in explaining the method of correcting, the low count dilution is referred to as dilution X and the high count dilution as dilution Y. If a given sample has an X dilution count that is higher than that at the neutral point, this count is used without correction. The Y dilution count on the

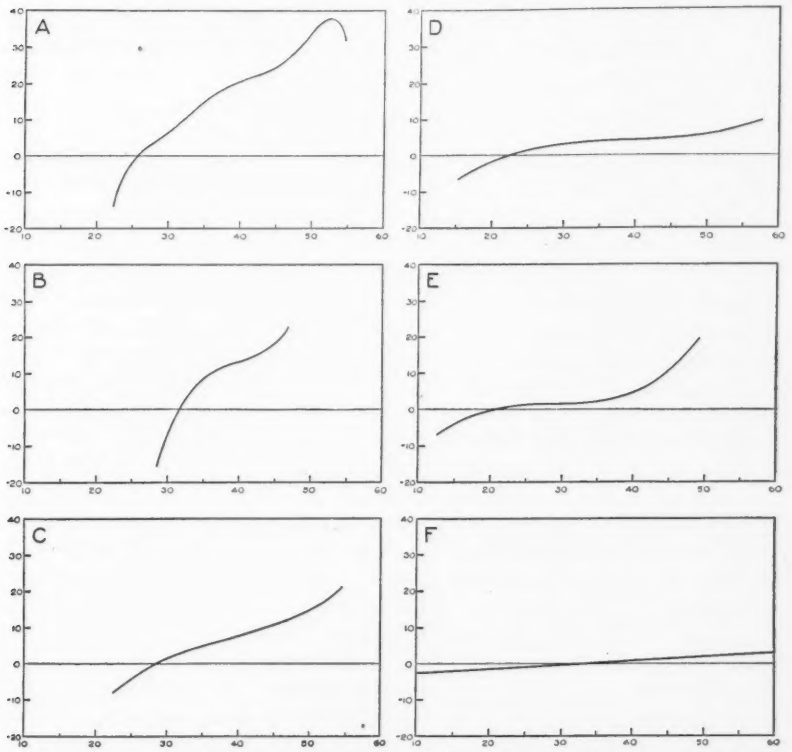

FIG. 2. *Correction curves for counts from two dilutions of different strengths, derived from the data of Fig. 1. The 0 line represents the theoreticals for y.*

same sample is corrected to make interchangeable with X dilution counts. To make the correction, find the X dilution count on the base line and at this point measure the difference from the curve for Y to the theoretical. Add the difference to the actual count for the Y dilution of the sample. The estimate on the sample is obtained by averaging that calculated from the actual count for dilution X and that from the Y dilution count, corrected as above. This correction lessens the error attributed to crowding in the plates with the larger number of colonies. Since it has been shown that counts from the X dilution are too low when less than that at the neutral point, the Y dilution count is used without correction in a sample that has a low X dilution count and the latter is corrected to make interchangeable with Y dilution counts. This involves the preparation of a second curve, where the high count dilution is considered as X and the low count dilution values are plotted. The procedure for correcting is the same as in the former case. Here, the correction lessens the error attributed to the lack of mutual stimulation among colonies in plates from the dilution giving the lower counts.

As a test of the effectiveness of this correction, the means of the counts in the original data from Experiment 3 were adjusted and analysis of variance was run on the corrected data. The result follows:

Source	D.F.	Variance	*F*
Replicate dilutions	720	7.7307	1.0530
Systematic order	720		
Interaction	1440	7.3414	

The variance for dilutions is reduced from 16.6258 shown on the uncorrected data to 7.7307, with a corresponding decrease in the *F* value. The *F* value on the corrected data compares favourably with that of 1.1077 shown previously from Experiment 1 and that of 1.1065 from Experiment 2. These results furnish strong evidence that the correction was appropriate and reduced differences deliberately introduced by the system of diluting to approximately those obtainable among dilutions of one strength.

Additional evidence that the factor to which this correction was applied had a tendency to mask real effects in the data may be demonstrated as follows. In Experiments 1, 2, and 3 after the correction was applied, the variance introduced by replicate dilutions is only 5 to 10% larger than that due to the interaction of dilutions and systematic order. Therefore, for purposes of illustration, the dilution variance may be used as error instead of the interaction. The 36 tests for each day's plating represent attempts to measure differences among replicate field samples. *F* values calculated by dividing the variance available for estimating the effect of field samples by the dilution variance as error, give the following results on the original data and on the same data after the correction was applied.

Original data	Corrected data	
1.6458	5.0340	
2.2376	13.4925	
.6769	6.4460	
.9241	4.7326	
1.2544	5.7404	
1.1913	6.2913	For 35 and 72 D.F.
1.5711	4.3419	
1.4273	7.7981	5% Pt. 1.59
3.9320	8.6872	1% Pt. 1.92
2.6615	14.3237	

It may be observed that in the original data only one value is over that at the 5% level of significance and three over that at the 1% level of significance, while the lowest value in the corrected data is more than double that at the 1% level of significance. Consequently, when the data are corrected to the point where estimates for the three dilutions are interchangeable, the possibility of finding differences among field samples is much greater. Thus, this

correction reduces the error pertaining to the number of colonies per plate in the technique and makes the results obtained considerably more sensitive as potential indicators of differences among samples.

Discussion

The finding that a high count, although well within the range of accepted numbers of colonies per plate, introduces an error in the estimate that is greater than that of a low count from a dilution of the same strength further complicates the problem of evolving a satisfactory technique for determining the number of bacteria in soil. It indicates that the number of colonies that grow in a limited quantity of medium and under a definite set of conditions is controlled by what may be termed a plate factor error. A count of 30 may be subject to a plate factor error that is of minor significance; one of 40 to an error that is more serious and one of 80 to one that is more than double that of the 40 count. This makes it apparent that with counts of the order of 100 per plate an effect under consideration may be masked by the plate factor error to a greater extent than with counts of 40.

While the result obtained was limited to the mixed population developing from soil of one area under the conditions of the experiment, it is conceivable that the same phenomenon may apply in relation to counts of bacteria in other products. For example, a similar test might show that a count of 300 colonies from one dilution of milk may not be more than half what it would have been had the dilution used given a count of 30 colonies per plate. This would mean that the sample with the higher number of colonies per plate has the advantage in a product whose quality is measured in terms of low counts.

Likewise, the failure of two dilutions of different strengths, although such dilutions provide data relatively similar in the light of accepted ranges in counts, to provide estimates that check presents a problem of serious concern to one interested in estimating the soil population for the purpose of studying the relation of numbers of bacteria to conditions or treatments in terms of recognized statistical significance. Since the proper dilution for a given soil under one set of conditions is not predictable, the use of two dilutions of different strengths not only allows for the application of the correction suggested, but provides two chances of obtaining counts within the range most desired. This does not mean that the dilutions should differ widely. Undoubtedly, a wide range in dilutions would introduce the effect of extremes in either low or high counts or both. Neither should the dilutions represent those giving counts too closely alike, since it is conceivable that the disturbance resulting from the number of colonies per plate would be similar in both dilutions. The use of dilutions yielding counts varying as 1 : 2 or 1 : 3 would seem to be most desirable. Under the writers' conditions, the dilutions giving the lowest number of colonies should be such as to provide counts above 25 per plate. The regression line for the 1 : 500,000 dilution counts as y and the 1 : 400,000 counts as x appears to provide further information on the effect of too low counts per plate. In all other cases, the lower dilutions, with

higher counts than those of the 1 : 400,000 used as x, give counts that become increasingly lower than the theoretical as the size of the counts increases. In this instance, the opposite appears to be the case. The higher dilution with the lower actual counts produces results that are lower than the theoretical. The fact that the 1 : 500,000 dilution counts may be too low on the basis of the theoretical may provide an explanation for the relatively small correction shown for the 1 : 300,000 counts when the 1 : 500,000 dilution counts are used as x.

The relation of the number of colonies that grow in a plate to the probable number of organisms actually in the portion measured provides reason for considering the influence of associative action among types of organisms on the number of colonies that develop. In the low count plates, the organisms depend on the food ingredients provided, while in the medium count plates, conceivably, the organisms may be affected by stimulation from the products released by other colonies near by and as well, by probable antagonistic relations. In crowded plates, because of the proximity of colonies and the greater probability of the presence of antagonistic types, the suppressing effect of antagonism may mask any stimulative effect caused by other colonies and result in a count much lower than the theoretical.

The correction suggested may find application in the study of antagonism between definite species of micro-organisms. The result of antagonistic relations could be studied quantitatively by making estimates of the two species developing separately and of the two in different proportions developing together on plates. Thus, it would be possible to measure the relative antagonistic effect of different species against one test organism.

Finally, the use of dilutions providing two ranges of counts and the correction suggested provides a method for estimating a population with a greater degree of accuracy than could result from the use of one dilution only. This procedure involves the replication of dilutions, with added labour, but results in an estimate of greater value without increasing the number of plates prepared. Three plates from each of two dilutions of different strengths would provide the basis for an estimate of greater value for the population considered than would six plates prepared from one dilution.

Acknowledgments

The authors are pleased to express thanks to Dr. C. H. Goulden, Senior Agricultural Scientist, Dominion Rust Research Laboratory, Winnipeg, for assistance with the statistical part of this study and for helpful criticisms in the preparation of the manuscript; and to Mr. A. M. Brown, Assistant Plant Pathologist, of the same laboratory, for photographing the illustrations.

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THE INFLUENCE OF LIGHT AND CERTAIN OTHER ENVIRONMENTAL FACTORS ON THE MATURE-PLANT RESISTANCE OF HOPE WHEAT TO STEM RUST[1]

By T. Johnson[2] and Margaret Newton[3]

Abstract

Experiments have been conducted to determine the degree to which the mature-plant reaction of Hope wheat to race 21 of *P. graminis Tritici* Erikss. and Henn. is affected by variation in light intensity, length of daily light period, temperature, soil moisture, and supply of mineral nutrients. All these factors gave evidence of influencing rust reaction. A 60% reduction in light intensity during the whole growing period of the plants tended to influence their rust reaction in the direction of increased susceptibility and caused a modification in the morphology of the plants in that they were softer, less rigid, and higher in moisture content than plants grown in full daylight. The shift towards susceptibility was, however, not very marked, and in only one experiment did the reaction of the plants approach complete susceptibility. Reduction in length of daily light period also influenced the rust reaction of the plants towards susceptibility. Plants receiving six hours of light daily were less resistant than plants receiving ten hours of light and these, in turn, were slightly less resistant than plants receiving full daylight for the period of the experiment (February to June). Experiments on the effect of temperature on the reaction of Hope wheat have shown that constant high temperature (75° to 80° F.) is capable of causing a partial or even complete breakdown of mature-plant resistance. Some evidence was secured that abundance of soil moisture and of mineral nutrients diminished rust resistance.

Introduction

In recent years the varieties Hope and H 44-24 have been utilized to a great extent in crosses designed to develop rust-resistant varieties of common wheat. These varieties, though susceptible to many physiologic races of stem rust in the seedling stage, appear to be highly resistant to most—if not all—races in the mature-plant stage. This type of resistance has frequently been termed "mature-plant" resistance. As the new varieties developed through crosses with Hope and H 44-24 also exhibit this type of resistance, it is obviously of much importance to determine its stability under the various environmental conditions to which these new varieties will inevitably be subjected when they become widely distributed. The necessity for a study of this kind is emphasized by the fact that varieties possessing mature-plant resistance do not by any means exhibit the same degree of resistance in all regions where they, thus far, have been grown.

On the North American continent varieties with mature-plant resistance have, in general, proved highly resistant. Abbott (1), however, has reported that, when grown in Peru, South America, on irrigated soil, Hope and Pentad

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rusted heavily. Similarly, when grown in Kenya, East Africa, varieties with mature-plant resistance have shown considerable rust development (2, 3). The possibility that the susceptibility of these varieties is due to the presence of physiologic races differing from those prevalent in North America cannot be entirely overlooked. In Kenya, however, where surveys of physiologic races have been conducted annually, three of the four races known to exist when these wheats were tested, races K_1, K_2, and K_3, are identical with races 21, 17, and 34, to which Hope, H 44, and their derivatives are highly resistant in Canada and the United States (9).

While it is believed that the environment in the above-mentioned regions has greatly influenced the stem-rust reactions of these wheat varieties, it is not at all clear what factors in the environment have exerted the greatest influence. The two localities in which these tests were conducted, Lima, Peru, and Njoro, Kenya, do not appear to have many environmental conditions in common except that, as both are located close to the equator, day and night are approximately equal in both places. At Lima the altitude is low, about 500 ft., the rainfall negligible so that wheat must be irrigated, while fogs and mists are common during the period when wheat is grown. At Njoro the altitude is high, about 7,100 ft., while the annual rainfall varies between 30 and 50 in. In both localities temperatures are moderate during the growing season for cereals.

As suggested by Burton (*cf.* Hart and Zaleski (5, p. 1044)), altitude is probably of little importance except in so far as it modifies other environmental conditions. The approximate equality of day and night may be of some importance in modifying plant growth and reaction to rust. Whatever the conditions responsible, it is, at any rate, evident that in Kenya the growth of wheat is much slower than in Canada. Hope wheat sown on April 24, 1935, was not harvested until October 7—166 days after sowing*. An average for a period of six years showed that, at Winnipeg, the same variety matures in 90 days. In Peru, the fact that the plants were grown under conditions of irrigation, accompanied by fogs and mists during the growing period, may have played a part in breaking down mature-plant resistance.

Hart and Zaleski (5) have already studied the effect of light intensity and temperature on the reaction of Hope wheat to stem rust. They have found that, under reduced intensity of light, Hope wheat becomes susceptible to race 21 of wheat stem rust. High temperatures (26° to 33° C.) on the contrary tended to increase the resistance of the plants. Apart from their work no serious attempts appear to have been made to evaluate, by experimental means, the relative influence of different factors of the environment on mature-plant resistance.

The experiments described in the present paper were conducted with the object of discovering, if possible, the relative importance of such environmental factors as intensity of light, duration of daylight, temperature, soil

* *Information furnished privately by Mr. R. J. Lathbury, Plant Breeding Station, Njoro, Kenya*

moisture, and supply of soil nutrients, in breaking down mature-plant resistance. The experiments were carried out in the greenhouse as these factors could be more readily controlled under greenhouse conditions than in the field.

Method of Estimating Degree of Resistance

In the work reported in the present paper it was essential to secure as accurate a comparison as possible of the resistance to rust of a given variety grown under different environmental conditions. In most of the experiments the infection types occurring on the plant were recorded in detail, leaf by leaf and internode by internode. As different parts of the same plant commonly bore infections of two or more types, difficulties were often experienced in deciding on an infection type that could be considered characteristic of the variety under a given environmental condition. An attempt was made to overcome these difficulties by utilizing the following scale of numerical values assigned by Goulden, Newton, and Brown (4) to the various infection types originally described by Stakmen and Levine (13).

Numerical values	1	2	3	4	5	6	7	8
Infection types	0	0;	1=	1−	1	1±	1+	1++
Numerical values	9	10	11	12	13	14	15	16
Infection types	2=	2−	2±	2+	2++	3=	3−	3
					x−	x	x+	x++
Numerical values	17	18	19	20	21	22	23	24
Infection types	3±	3+	3++	4=	4−	4	4+	4++

By assigning numerical values to all the infection types recorded for a plant or a group of plants of the same variety, it was possible to arrive at a mean numerical value indicative of the average rust reaction of the variety. Resistance is indicated by numerical values below 13 and susceptibility by values above 16. The intermediate values 13 to 16 represent the transition from resistance to susceptibility.

The Effect of Reduced Light Intensity on the Reaction of Hope Wheat to Race 21 of Stem Rust

Three separate greenhouse tests were carried out to determine the effect of reduced light intensity on rust development on the variety Hope. The first of these was conducted in the spring (March and April) of 1936, the second in the early winter (November and December) of the same year, and the third in the spring of 1937 (April and May). The conditions of light were approximately the same in the first and third tests, but in the second the day was shorter and the light intensity lower.

The reduction of light intensity was effected by growing the plants from the seedling stage to maturity under a covering of cheesecloth, one ply being used in the first test and two in the second and third. After inoculation, the plants were again exposed to the light conditions under which they were grown.

The same race of stem rust, race 21, was used in all three trials. As the shaded plants used up less moisture than the non-shaded, attempts were made, in all the experiments, to equalize soil moisture in the shaded and non-shaded pots by watering the two lots in such a way that the soil in both maintained about the same appearance and handling qualities.

In the first test ten 6-in. pots, each containing three plants, were placed under a one-ply cheesecloth cover soon after the emergence of the seedling plants, while ten pots were kept in the open as controls. About seven weeks after planting, the plants in two pots of each group were inoculated with race 21 by applying the urediospores to the leaf and stem surfaces with the fingers. Thereafter, at intervals of about ten days, two pots were removed from each of the two groups and the plants inoculated in the same manner. The first inoculations were performed on March 13, when the plants were in the early shooting stage, the last on April 24, when the plants were heading.

TABLE I

THE EFFECT OF REDUCED LIGHT INTENSITY ON THE REACTION OF HOPE WHEAT TO *P. graminis Tritici*, RACE 21

The wheat was planted January 24, 1936, and inoculated at different dates between March 13 and April 24. (The upper half of the table shows the degree of resistance in terms of infection type; the lower half shows the degree of resistance in terms of numerical values calculated from the scale developed by Goulden, Newton, and Brown.)

Date of inoculation	Date of marking	Leaves		Stems	
		Shaded	Not shaded	Shaded	Not shaded
13-3-36	27-3-36	2 to 4=	2 to 3	2 to 4−	3
24-3-36	11-4-36	2 to 4−	0; to 4	3±	3 to 4
3-4-36	23-4-36	1 to 3=	2 to 3	2 to 3	2 to 3−
17-4-36	2-5-36	1 to 2	1 to 2	2 to 3+	1 to 3
24-4-36	8-5-36	0; to 1+	0; to 2	1 to 3	1 to 3
13-3-36	27-3-36	15.4	13.7	16.3	16.0
24-3-36	11-4-36	16.0	13.2	17.0	19.0
3-4-36	23-4-36	9.5	13.5	13.5	12.6
17-4-36	2-5-36	8.0	7.5	14.4	10.6
24-4-36	8-5-36	4.2	6.0	10.5	10.2
Mean of all tests		10.6	10.8	14.3	13.7

The results of this test, which are expressed in Table I, do not reveal any appreciable difference in the type of infection produced by race 21 under the two different conditions of light. Under both full and reduced light there was a progressive increase in the resistance of both leaves and stems with a progressively later date of inoculation. This is probably due mainly to the increasing age of the plants, as each successive batch of plants was about 10 days older than the preceding one. The possibility, however, is not altogether excluded that the increasing length of day and intensity of daylight may have played some part in the development of greater resistance.

As it was thought possible that the failure of the first test to show any relation between light intensity and rust reaction might have been due to an insufficient reduction of the intensity of light, it was decided to conduct a somewhat similar experiment with Hope wheat at a time of year when the intensity of daylight was lower, and with the plants shaded by two ply of cheesecloth instead of one as in the earlier test.

Twenty-four 6-in. pots planted on September 4 were divided into two equal lots, one of which was exposed to natural daylight conditions while the other was placed in a cage covered by two ply of cheesecloth. Between November 21 and 26, when the plants were in the boot stage, they were inoculated with race 21. Following inoculation the shaded plants were again placed under their cheesecloth cover. Notes made on rust development on December 11 are summarized by means of numerical values in Table II.

TABLE II

THE EFFECT OF REDUCED LIGHT INTENSITY ON THE REACTION OF HOPE WHEAT TO *P. graminis Tritici* RACE 21

The wheat was planted September 4, 1936, and inoculated between November 21 and 26

Pot	Reaction in terms of numerical values for infections			
	Leaves		Stems	
	Shaded	Not shaded	Shaded	Not shaded
1	10.5	13.5	16.0	16.5
2	14.5	—	16.5	13.5
3	16.0	13.5	16.5	13.5
4	16.0	5.0	13.5	11.5
5	16.0	—	16.5	10.0
6	14.5	11.0	14.5	10.5
7	16.0	13.5	16.0	8.0
8	13.5	13.5	13.0	10.5
9	16.5	19.0	18.0	13.5
10	13.5	—	16.5	10.0
11	10.0	14.5	16.0	11.5
12	13.5	16.5	16.5	13.5
Mean	14.2	13.3	15.8	11.9

It was obvious from a cursory examination that the shaded plants were somewhat more susceptible than the non-shaded. The percentage of infection was also considerably higher on the shaded plants: the average of the estimates for the individual plants were 5.4% on the leaves and 11.3% on the stems of the shaded plants as against 0.7% and 6.0% for the leaves and stems of the non-shaded plants. Although the shaded plants were noticeably more susceptible than the non-shaded ones, the average increase in susceptibility was not very striking. Translated from numerical values to infection types, it amounted to a shift from 2++ and x− to 3= and x on the leaves; and from 2+ to 3 and x++ on the stems.

The shaded and non-shaded plants differed not only in their reactions to rust but also in certain other characteristics. The non-shaded plants were harsher to the touch and considerably more rigid in both stem and leaf than the shaded ones; this gave the impression of somewhat greater succulence in spite of their more slender culms. This observation led to a determination of the moisture content of the two sets of plants. The moisture content of the shaded leaves was 82.68% as against 76.62% for those of the non-shaded; and of the shaded stems, 85.46% as against 81.59% for the non-shaded.

It can scarcely be doubted that the increased succulence and the greater susceptibility of the shaded plants are related phenomena. There is some doubt, however, as to whether or not the greater succulence of the shaded plants was entirely the result of the lower light intensity at which they were grown. It should be noted that the relative humidity within the cheesecloth cage averaged almost 10% higher than outside. Nevertheless the difference in relative humidity inside and outside the cage was slight as compared with the difference in light intensity. Measurements with a Weston Photronic Exposure Meter showed that, under conditions of diffuse daylight, the double ply of cheesecloth transmitted only from 35 to 40% of the light. As the experiment was conducted in the fall and early winter, even the plants receiving

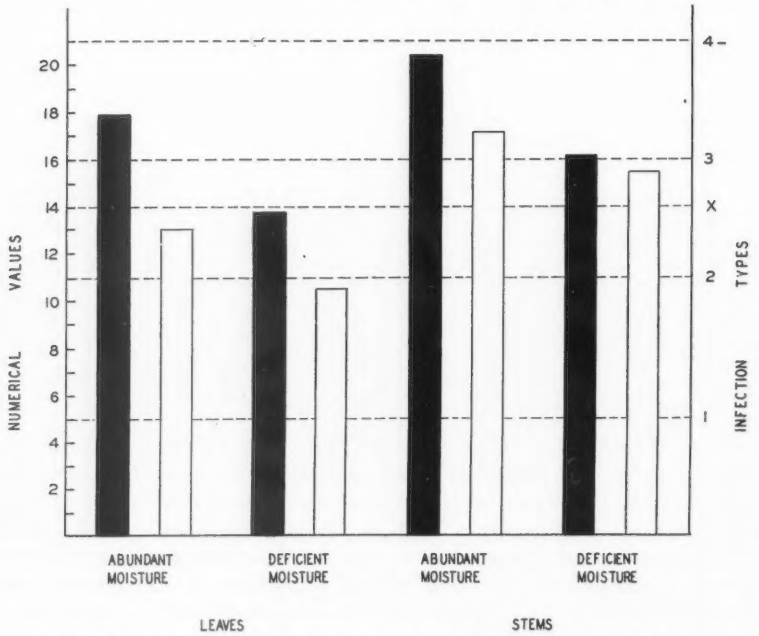

FIG. 1. *Average rust reactions of shaded and non-shaded plants of Hope wheat grown under conditions of abundant and deficient soil moisture. The black columns represent shaded, the white columns, non-shaded plants. The plants were inoculated during the third week of April.*

full daylight grew under distinctly subnormal light conditions. Despite the still lower light intensity received by the shaded plants, their resistance to race 21 was broken down to only a rather limited extent.

A third experiment of a similar type was carried out in the spring of 1937, the plants being sown late in January. Twenty-four pots containing three plants each were divided into two groups one of which was shaded by two ply of cheesecloth while the other was exposed to ordinary daylight. This experiment, however, differed from the previous ones in that one-half of the shaded and non-shaded plants, respectively, were grown under conditions of deficient soil moisture. The purpose of this modification was to reduce the succulence of both shaded and non-shaded plants and determine the relation of such reduction in succulence to rust reaction.

The plants were inoculated with race 21 at the time of the emergence of the heads, in the third week of April. After inoculation each group of plants was re-exposed to its previous conditions until notes were taken on rust reaction. The results of this experiment are expressed in Table III and Fig. 1.

TABLE III

THE EFFECT OF REDUCED LIGHT INTENSITY ON THE REACTION OF HOPE WHEAT TO RACE 21 OF *P. graminis Tritici* UNDER CONDITIONS OF ABUNDANT AND DEFICIENT MOISTURE

The wheat was planted late in January, 1937, and inoculated in the third week in April

Treatment	Pot	Reaction in terms of numerical values for infections			
		Leaves		Stems	
		Shaded	Not shaded	Shaded	Not shaded
Plants supplied with abundant moisture	1	18.5	14.0	19.0	18.0
	2	17.0	15.0	19.0	17.0
	3	19.0	13.0	21.0	17.5
	4	19.0	11.0	21.0	15.0
	5	17.0	—	21.0	20.0
	6	17.0	12.0	21.0	15.0
Mean		17.9	13.0	20.3	17.1
Plants subjected to drought	7	13.5	6.0	17.0	17.0
	8	16.0	12.0	17.0	15.5
	9	16.0	11.0	18.0	16.0
	10	11.0	11.0	13.5	15.5
	11	13.0	11.0	17.0	15.5
	12	13.5	12.0	15.0	13.0
Mean		13.8	10.5	16.2	15.4

The effect of shading on the plants, and particularly those receiving abundant moisture, was very similar to that noted in the preceding experiment. Moisture determinations on leaves and stems of the plants receiving abundant moisture showed, at the finish of the experiment, about two weeks after heading, that the shaded leaves contained 77.93% moisture as against

72.20% for the non-shaded leaves, while the shaded stems contained 77.36% moisture as against 67.82% for the non-shaded.

The infection results, as summarized in Fig. 1, show that both light and moisture were capable of affecting the rust reactions to some extent. In general, the susceptibility of both leaves and stems was somewhat greater than in previous experiments. Resistance was greatest under conditions of full light plus deficient moisture, whereas susceptibility was greatest under conditions of reduced light plus abundant moisture. Under the latter conditions the plants would have to be described as moderately susceptible. The relation between succulence and susceptibility was quite evident.

The Relation of Light to Regional Susceptibility

It is possible that light conditions may account for the "regional susceptibility" of varieties possessing mature plant resistance to which attention has been called by Goulden *et al.* (4), Popp (12), and Newton and Brown (11). It is characteristic of varieties with mature-plant resistance that the growing plant tissues are susceptible to rust while still covered by the sheaths, but acquire resistance very soon after they become exposed. Before they become exposed, these tissues are protected from light to a certain extent by the overlying sheaths. An attempt was made to gauge the extent of this protection by determining by means of a Weston Photronic Exposure Meter the proportion of diffuse daylight transmitted by freshly detached sheaths of wheat plants approaching the boot stage of growth. The sheaths were found to transmit from 25 to 33% of the light. Under identical light conditions, the two-ply cheesecloth used for shading plants transmitted 40% of the light while the cotton cloth used in some experiments for the same purpose transmitted 29%. It would seem therefore that the sheaths afford much the same degree of protection from light as the materials used for shading plants in the experiments reported in this paper. Whether the protected parts are susceptible because they are shaded from light, or because of the higher humidity within the sheath, or for other reasons, does not appear to have been proved experimentally. As it has been shown by Johnson and Johnson (7) that the immature tissues within the sheaths are very much richer in reducing sugars than are the fully developed tissues, and as these sugars may be regarded as the structural units for further tissue development, it is quite possible that the greater susceptibility of the less mature tissues is related to their stage of development rather than to deficiency in light.

'An Experiment to Determine the Effect of Different Periods of Daylight on Stem-rust Development on Hope Wheat

The idea of carrying out an experiment on the effect of different periods of daylight on mature-plant reaction was suggested by the results of certain tests in Kenya, East Africa, of wheat varieties developed at the Dominion Rust Research Laboratory. These varieties, which in Western Canada exhibited a high degree of mature-plant resistance, showed a considerable

degree of susceptibility when grown at Njoro in Kenya, in 1935. Environmental conditions in Kenya are, of course, strikingly different from those prevailing in Western Canada. As Njoro is on the equator, where day and night are approximately equal, the period of daylight is considerably shorter than that prevailing in Western Canada during the growing period where the period of longest daylight exceeds 16 hr. As a result of the shorter period of daylight, and perhaps other conditions as well, the growing period for wheat in Kenya is at least two months longer than in Western Canada.

It was thought possible that a greenhouse experiment, in which both temperature and period of daylight were kept under strict control, might produce information on the effect of length of day on mature-plant resistance. Accordingly, the variety Hope was grown from seedling stage to maturity under conditions of three different periods of daylight: a 6-hr. period, a 10-hr. period, and the full period of daylight during the course of the experiment. The last-mentioned varied considerably as the experiment was begun at the beginning of February and was not completed until June. As it was essential to keep conditions other than light identical for all the test plants, these were

FIG. 2. *The effect of different periods of daylight and of different temperatures on the development of Hope wheat. The photograph was taken about three months after planting.* 1—*10 hr. light daily; 60 to 61° F.* 2—*6 hr. light daily; 60 to 61° F.* 3—*Normal daylight; 10 hr. daily at 60 to 61° F. and 14 hr. at 75 to 80° F.* 4—*Normal daylight; 60 to 61° F.* 5—*Normal daylight; 75 to 80° F.*

grown (in 6-in. pots) in the same greenhouse, maintained at a temperature of 60° to 61° F. The plants exposed to daylight for 6 and 10 hr. respectively were kept during the remainder of each day in a dark chamber (in the same greenhouse) through which a gentle current of air was drawn by means of a fan to prevent the temperature and humidity within the chamber from rising above that of the greenhouse. In spite of this precaution, the temperature within the chamber averaged from 2 to 3° F. higher than that outside, but the relative humidity remained approximately the same as outside.

The effect of the 6- and 10-hr. periods was expressed in an abnormally slow rate of growth and a partial assumption of a winter habit (Fig. 2). Curiously, the rate of growth was slower for the 10-hr. than for the 6-hr. plants. The plants were all infected at the same stage of growth, at the time of the emergence of the head, but, owing to the different rates of growth in the three lots of plants, it was not possible to inoculate them all at the same time. The plants subjected to full daylight headed and were inoculated with race 21 in the second week of May; those exposed to 6 and 10 hr. of daylight were inoculated about six weeks later, on June 19.

The infection results are given in Table IV. It is clear from an examination of Table IV that the plants in all three groups had acquired mature-plant resistance by the time they were infected, in spite of the radically different conditions of light to which they were subjected. This resistance was greatest in the plants grown in full daylight and least in those exposed to only six hours of daylight. It would seem, therefore, that while the effect of a reduced period of daylight is definitely in the direction of increased susceptibility, there is no real inhibition of the capacity of the plant to develop mature-plant resistance.

TABLE IV

THE EFFECT OF DIFFERENT PERIODS OF DAYLIGHT ON THE REACTION OF HOPE WHEAT TO RACE 21 OF *P. graminis Tritici*

The wheat was planted February 1

Pot	Reaction in terms of numerical values for infections					
	Leaves			Stems		
	6 hr. daylight	10 hr. daylight	Full daylight	6 hr. daylight	10 hr. daylight	Full daylight
1	11.5	4.0	—	14.5	8.0	11.0
2	6.6	9.0	2.0	13.7	10.0	—
3	12.0	10.0	2.0	14.1	11.7	14.5
4	13.3	9.5	—	17.5	7.4	5.3
5	10.7	8.3	6.0	14.6	8.4	9.7
6	7.2	7.3	2.0	14.6	9.1	7.0
7			—			4.7
8			4.2			11.0
9			8.5			6.2
Mean	10.2	8.0	4.1	14.8	9.1	8.7

The Effect of Temperature on the Reaction of Hope Wheat to Stem Rust

Simultaneously with the previously described experiment on the effect of different light periods on rust development, another experiment was conducted to test the effect of temperature on the reaction of Hope to race 21 of stem rust. The test plants were grown to maturity under three different conditions of temperature: (i) a constant low temperature (60° to 61° F.), (ii) a constant high temperature (75° to 80° F.), and (iii) a combination of these (10 hr. daily at the low and 14 hr. at the high temperature). The plants were grown in 6-in. pots, three plants per pot, and ten pots to each of the three different temperature groups. The plants were inoculated at the time of heading. As certain of the plants grown at constant high temperature headed slightly in advance of the others, these were inoculated about four days earlier than the rest. The infection results are summarized in Table V.

TABLE V

THE EFFECT OF TEMPERATURE ON THE REACTION OF HOPE WHEAT TO RACE 21 OF *P. graminis Tritici*

Pot	Reaction in terms of numerical values for infections					
	Leaves			Stems		
	61–61° F.	10 hr. 60–61° F. 14 hr. 75–80° F.	75–80° F.	60–61° F.	10 hr. 60–61° F. 14 hr. 75–80° F.	75–80° F.
1	—	4.5	4.0	11.0	—	—
2	2.0	—	—	—	9.0	—
3	2.0	—	5.0	14.5	—	15.0
4	—	2.0	—	5.3	10.5	—
5	6.0	6.0	8.0	9.7	8.9	13.3
6	2.0	—	4.7	7.0	10.2	11.8
7	—	4.0	7.5	4.7	8.5	14.0
8	4.2	—	5.3	11.0	10.0	13.3
9	8.5	—	4.7	6.2	9.0	9.0
10		8.0			4.3	
Mean	4.1	4.9	5.6	8.7	8.8	12.7

The tabular representation of the infection results shows that the plants in all three groups exhibited considerable resistance which was, however, least pronounced in the plants subjected to continuous high temperature. The reactions of the plants grown at low and at alternating low and high temperature were almost identical.

In a later test the breakdown of the resistance of Hope wheat at constant high temperatures was very pronounced. Plants grown and kept after inoculation at high temperatures (75° to 80° F.) developed infections averaging, in numerical values, 16.5 on the leaves and 15.8 on the stems, whereas plants grown and kept after inoculation at ordinary greenhouse temperatures

(55° to 80° F. daily) developed infections averaging 5.4 on the leaves and 7.5 on the stems. This represents a shift in reaction from resistance (infection types "1− 2=") at ordinary temperatures to susceptibility (infection type "3+") at high temperatures.

The Effect of Fertilizer on the Reaction of Hope Wheat to Stem Rust

An experiment designed to test the effect of fertilizer on the rust reaction of Hope wheat under conditions of reduced light intensity was conducted in the spring of 1939. This combination (the presence of fertilizer plus reduced light intensity) was thought conducive to the breakdown of mature-plant resistance. The plants were grown under one ply of cotton cloth that transmitted about 30% of light when tested with a Weston Photronic Exposure Meter in diffuse daylight. All plants were grown in 6-in. pots with three plants in each pot, one-half being grown in very light soil (one-third soil, two-thirds sand) which received no fertilizer, the other half in similar soil supplied monthly with a full nutrient solution (Shive's solution). All plants were inoculated with race 21 of *P. graminis Tritici* when the heads were beginning to emerge. Inoculation took place at the end of May and notes were taken on rust infection on June 12.

In this experiment there was very little difference in the reaction of the plants that received fertilizer and those that did not, despite the fact that the former were distinctly more succulent. The plants grown in non-fertilized soil were rather highly resistant; those grown in fertilized soil moderately resistant (Table VI). In no instance was there any breakdown in mature-plant resistance, a fact which suggests that this type of resistance is not readily influenced by the application of fertilizer.

TABLE VI

THE EFFECT OF FERTILIZER ON THE REACTION OF HOPE WHEAT TO RACE 21 OF *P. graminis Tritici*

Pot	Reaction in terms of numerical values for infections			
	Leaves		Stems	
	Fertilizer present	Fertilizer absent	Fertilizer present	Fertilizer absent
1	7.0	5.3	5.6	7.2
2	5.0	5.0	8.0	5.0
3	8.0	5.0	9.2	5.0
4	5.0	5.0	9.0	5.0
5	8.0	—	8.2	6.6
6	8.0	—	10.3	4.7
7	10.0	8.5	8.8	8.0
8	8.5	—	10.0	—
9	—	—	6.5	—
10	7.5	7.0	10.8	8.0
Mean	7.4	6.0	8.6	6.2

Discussion

Although mature-plant resistance occurs in many new wheat varieties derived from crosses with Hope or H 44, its nature is as yet little understood. Hart and Zaleski (5) have shown that this type of resistance finds its greatest expression under conditions of high light intensity while low intensity of light may result in its breakdown to a greater or less degree.

Although some of the experiments reported in this paper have confirmed the above-mentioned conclusions, it should be emphasized that the shading experiments thus far conducted have not consistently resulted in such a breakdown. The lack of consistent results in these experiments is puzzling. If the mature-plant resistance of Hope is fundamentally conditioned by light, it would be expected that drastic reduction in light intensity would always result in marked increase in susceptibility to rust. Such does not seem to be the case. Plants of Hope and other varieties with the same type of resistance have frequently been inoculated in the greenhouse during midwinter, when solar radiation is less than one-quarter of that of midsummer, and the amount of light received by the plants is frequently still further reduced by heavy coatings of frost on the roof of the greenhouse. Under such unfavourable light conditions it might be expected that these varieties would invariably show a certain degree of susceptibility, yet plants infected in midwinter often show rather high resistance.

In the two experiments in which shading resulted in increased susceptibility, there was a rather marked modification in the growth of the shaded plants. They were softer to the touch and noticeably less rigid than the plants grown in full light. Dry weight determinations showed that the moisture content of both stems and leaves was considerably higher in the shaded plants. It is likely that the increased succulence of the shaded plants is mainly a consequence of the low light intensity at which they were grown, although the somewhat higher humidity inside the cheesecloth cages may also have contributed. As the shading of the plants raised the humidity by only about 10% whereas it decreased the light intensity by 60 to 70%, it is likely that the greater share of the increased succulence and susceptibility of the shaded plants should be attributed to the low intensity of light under which they were grown. Possibly reduced light intensity is capable of increasing the susceptibility to rust only when other growing conditions are such as to lead to an increase in the moisture content or succulence of the plants.

As mentioned elsewhere in this paper, mature-plant resistance, although generally effective in North America, has been known to break down in other parts of the world, such as Peru, South America, and Kenya, East Africa. In view of the fact that the daily period of light is shorter in these equatorial regions than it is during the wheat growing season in Canada and the United States, it was thought possible that Hope plants grown in the greenhouse under short day-length conditions might be more susceptible to stem rust than plants grown under conditions of longer day-length. The experimental evidence obtained tends to support this idea, although even the plants grown under

conditions of only six hours daylight per day appear to possess a certain degree of resistance. The plants grown in 10 hr. of daylight and those grown in full daylight were, however, considerably more resistant. The reduction in the daily period of light had the effect of forcing the plants for a while into a semblance of winter habit. When, however, the plants finally emerged from this growth habit and came into head they appeared to have acquired a certain degree of mature-plant resistance in spite of the short day-length. Nevertheless the experimental results do suggest that plants grown under conditions of short day-length are less highly resistant than plants grown under longer daylight periods.

The reduction in light intensity required to shift the reaction of Hope wheat from resistance to moderate susceptibility is so great that it is difficult to imagine that low light intensity could be the main factor in causing breakdown in mature-plant resistance in nature. In view of the experiment on the influence of day-length on the rust reaction of Hope, it is equally difficult to explain the breakdown of mature-plant resistance in equatorial regions as merely the effect of a shorter period of daylight. The plants grown in 10 hr. of daylight—a period shorter than the day-length of tropical regions—were rather highly resistant. Where the light period was reduced to six hours per day there was, however, a partial breakdown of resistance. As far as can be judged from this experiment, therefore, a daylight period such as that prevailing in Peru or Kenya would, of itself, be insufficient to account for the reaction of Hope and similar varieties in these regions.

Three other factors were considered in this paper, namely, soil moisture, soil nutrient supply, and temperature. The experimental results indicate that increase of soil moisture and supply of soil nutrients increase the succulence of the plant and thereby its susceptibility. There is some evidence that the mature-plant resistance of Hope wheat can be broken down at high temperatures. The infection results tend to confirm the idea that progressively higher temperatures lead to a progressive increase in susceptibility (6, 10, 14). This increase, however, reaches its limits at abnormally high temperatures as shown by Johnson and Newton (8), and there must therefore be some temperature point at which susceptibility no longer increases but begins to diminish. The experiments on temperature reported in the present paper suggest that the susceptibility of Hope increases with rising temperature contrary to observations recorded by Hart and Zaleski (5). As they reported a temperature variation from 26° to 33° C. (79° to 91° F.), it is possible that, in their experiment, the temperature had reached a point beyond the maximum at which susceptibility continues to increase.

Despite such attempts as have been made to elucidate the nature of mature-plant resistance, it must be admitted that the problem is still far from being solved and, as yet, not even the influence of environmental conditions on the expression of resistance is fully understood. There can be little doubt that conditions of light do influence rust reaction, but so also do conditions of temperature, moisture, and soil nutrients. The experimental results secured

in the work here described indicate that deficiency of light can increase susceptibility in Hope only in conjunction with other factors which influence plant development in the direction of increased succulence. It would appear that there is an inherent tendency in Hope to acquire resistance to rust as the tissues become mature, and that very subnormal light conditions, moderately high temperatures, and other factors exert a repressive influence on this process.

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INTERSPECIFIC AND INTERGENERIC CROSSES WITH *HORDEUM*[1]

By F. L. Quincke[2]

Abstract

Crosses are described between *Hordeum jubatum* (28 chrom.) × *Hordeum vulgare* (14 chrom.), *H. nodosum* (42 chrom.) × *H. vulgare*, *H. vulgare* × *Secale cereale* (14 chrom.), *H. jubatum* × *S. cereale*. In the interspecific crosses viable seeds were obtained which produced healthy and vigorous plants. The seeds of the intergeneric crosses did not germinate.

The enormous soil losses of the Western Plains due to wind and water erosion necessitates the production of winter hardy cereals which will cover the land during the critical period of the late winter and early spring. A few crosses have been made with the hope of developing a winter hardy barley for the Northwestern Plains.

Hordeum jubatum × *Hordeum vulgare hex. (var. Friedrichswerther)*

When pollen of the *H. vulgare* was transferred to the stigma of *H. jubatum* approximately 70% of the florets set seed. The F_1 seeds were slightly shrunken as compared with the selfed seed of *H. jubatum* and only 9% germinated. The plants developed somewhat slowly at first. After four weeks they produced as much top growth as the female parent. At heading stage the F_1 exceeded the female parent considerably in size and amount of foliage. The F_1 plants retained the perennial habit of the female parent but differed from the latter in having long auricles, short pubescence, well developed lateral florets, larger florets and shorter awns.

Hordeum jubatum × *Hordeum vulgare dist. (var. Rex)*

In this cross 78% of the pollinated florets set seed. Of the seed obtained, 54% produced strong and vigorous plants, of a height about twice that of the female parent. The pubescence of the female parent was lacking in the F_1 plants.

Hordeum nodosum × *Hordeum vulgare dist. (var. Hanna)*

Forty-three per cent of the florets pollinated with pollen of the variety Hanna set seed. Twenty-eight per cent of the seeds were viable. The F_1 plants showed considerable vigour as compared with the female parent. They also lacked pubescence.

[1] *Manuscript received February 6, 1940.*
Contribution from the University of Saskatchewan, Saskatoon, Sask.

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*Hordeum vulgare (var. Friedrichswerther) × Secale cereale (var. Crown)**

When pollen of *S. cereale* var. Crown was placed on the stigma of *H. vulgare* var. Friedrichswerther approximately 90% of the florets set seed. The matured seeds were very shrunken. When dissected, they showed no traces of embryo or endosperm. A detailed investigation after pollination revealed that while fertilization of the embryo and endosperm nucleus took place, the development of both soon ceased. Eight days after fertilization both endosperm and embryo had disintegrated.

Hordeum jubatum × Secale cereale (var. Crown)

Ninety-five per cent of the florets pollinated set seed. The seed was very shrunken and failed to germinate. When dissected it showed a well developed embryo sac without any traces of the embryo or endosperm.

* *The writer is much indebted to Dr. W. P. Thompson for his cytological study of the cross H. vulgare × S. cereale which was discussed by the latter in his paper on "The Frequency of Fertilization and the Nature of Embryo and Endosperm Development in Intergeneric Crosses in Cereals" given before the Genetic Congress in Edinburgh, 1939.*

NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

V. BIOLOGICAL ASSAYS OF HAY CROP RESIDUES[1]

By R. Newton[2] and R. S. Young[3]

Abstract

As part of a study of crop sequence effects, the possible toxicity of fermentation products of crop residues was investigated. Adding residues to soil samples caused stimulation rather than inhibition of nitrification rates in the laboratory. Extracts of the residues fermented for different periods were added to cultures of *Penicillium* and of wheat plants. Here both toxic and vitamin-like effects were noted, the latter being dominant except in extracts from residues at early stages of decomposition. Effects of both kinds were transitory, but probably significant, a conclusion supported by considerable specificity noted in influence on fungus flora of soil.

Introduction

Earlier papers in this series dealt with residual effects of alfalfa (*Medicago sativa* L.), brome (*Bromus inermis* Leyss.), timothy (*Phleum pratense* L.), and western rye grass (*Agropyron tenerum* Vasey), on wheat crops at Edmonton, Alberta, as indicated by nitrogen absorption of crops (6) and nitrate accumulation and numbers of soil micro-organisms (4); and attempted to explain these by the composition of the hay crop residues (7), and the oxidation-reduction potentials and carbon dioxide production of residues and soils (3). The investigations covered the eight seasons 1927–34. Quite early in their course it began to appear that differences in the composition of the roots and stubble of the four hay crops might not account wholly for the differences in their after-effects. Accordingly the writers decided to re-examine the contentious question as to whether toxic decomposition products of the residues influenced subsequent wheat crops, either directly or through the soil microflora.

The method of biological assay, that is, of determining the presence of an active principle by its effect on the growth or health of some organism, was applied to fresh and fermenting crop residues. The test organisms used in the assays were nitrifying bacteria (in unsterilized soil), pure cultures of a blue mould, *Penicillium cyaneo fulvum* Biourge*, and common wheat.

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* *Kindly identified for the authors by Dr. Charles Thom, Principal Mycologist, Bureau of Plant Industry, Washington, D.C.*

Crop Residues Used

Sods were dug in the spring of 1930, before new growth had begun, to a depth of 10 in. They were washed to remove the soil as thoroughly as possible, dried, and the roots and stubble carefully divided at the crown for separate analysis. For these biological assays, however, the roots and stubble, after grinding in a Wiley mill to pass a 1-mm. sieve, were combined again in equal parts, this being roughly the ratio of their occurrence in one-year-old sods—the only sods in the main experiment that had been analysed up to that time. The mixed residues thus prepared were stored in tightly closed cans for use as required during a period of about a year.

It was not possible to take sods in sufficient quantity from the main experimental block without interfering with the subsequent course of the experiment. Accordingly, sods were dug from other available blocks. Since the residues differed significantly in composition from those of the main experiment (7, Tables I and III), the corresponding information for those used in the biological assays is given now in Table I.

TABLE I

COMPOSITION OF ORGANIC MATTER IN MIXED RESIDUES USED IN BIOLOGICAL ASSAYS

Crop	Age of sod, yr.	Crude protein, %	Ether extract, %	Crude fibre, %	N-free extract, %
Alfalfa	5	18.5	2.2	40.4	38.9
Brome	6	17.2	2.4	32.0	48.4
Timothy	2	17.9	3.2	27.5	51.4
Western rye	2	15.1	3.1	35.0	46.8

	Reserve* carbohydrates, %	Ratio carbohydrate† to protein	Ratio crude fibre to N-free extract
Alfalfa	16.9	4.3	1.04
Brome	21.6	4.7	.66
Timothy	19.9	4.4	.53
Western rye	19.1	5.4	.75

** Reducing sugar as dextrose after 3 hr. hydrolysis of residues in 1% hydrochloric acid on boiling water bath.*

† Carbohydrate taken as sum of crude fibre and nitrogen-free extract.

The nitrogen levels in these residues are considerably higher than those found in the main experiment, and the difference in this respect between the alfalfa and the grasses is much less. The ratios of carbohydrates to protein are correspondingly lower. These were the characteristics of the residues found in the main experiment to be most closely related to observed sequence effects. "Reserve carbohydrates", the new quantity included in Table I, represent an attempt to assess the readily available energy values of the

residues. Spoehr concluded in his investigation of cacti (8) that hydrolysis of dried, ground tissue in 1% hydrochloric acid for three hours under a reflux condenser on a boiling water bath converted starch and other normal reserve materials to monosaccharides, without affecting the cellulose. This method was applied to hay crop residues. The quantities found will obviously be related to the previous history of the stands sampled, and it has been explained that the samples were taken from different blocks, not uniformly treated. The values must therefore be regarded as peculiar to these samples, and not as affording a representative comparison of the four crops. The same consideration applies, though in lesser degree, to all the data in Table I.

Nitrification in Soil in Presence of Crop Residues

The observation by many workers, referred to in Part I (6), that the addition of carbohydrates to soil, as in straw or grass residues, causes a temporary disappearance of nitrates by assimilation in the bodies of micro-organisms fermenting this material, as well as our interest in the possible occurrence of toxic decomposition products, led us to try first the effect of the residues in controlled nitrification experiments. The plan of the experiment was similar to that used by Wilson and Wilson (9) in comparing nitrification of corn and sorghum roots. Twelve sets of five flasks each, comprising a check containing 100 gm. of dry soil only, and four flasks in which 0.5 gm. of residues were incorporated in the soil, were incubated at 25° C., and nitrates determined periodically by the phenoldisulphonic acid method (2). The soil was fertile Edmonton loam, containing about 15% organic matter and 0.60% nitrogen; it was sifted to remove coarse fibre. The flasks were brought to optimum moisture content, taken as 50% of the water-holding capacity of soil + residue, with a solution of calcium nitrate of such concentration as to add nitrate nitrogen equivalent to 40 p.p.m. of dry soil. The calcium nitrate was added, not to accentuate the disappearance of nitrates, as Wilson and Wilson used it (their soil being much poorer initially), but to minimize the effect of variations, due to sampling errors, in the original nitrate content of the soil in different flasks. An extra set of flasks was set up and analysed at once to determine the initial concentrations.

The experiment was carried out a second time, using the residues in approximately equal parts by weight of nitrogen, with brome and timothy still at 0.5 gm. The initial concentration of nitrate nitrogen in the five flasks analysed at once ranged from 128 to 133 p.p.m.

The results of both experiments were essentially alike, and only the second set need be presented. It is clear from Fig. 1 that there was neither any inhibition by toxic decomposition products nor any disappearance of nitrates. On the contrary, all the residues stimulated slightly the production of nitrates.

It may be thought that 0.5 gm. residues in 100 gm. soil, which caused a substantial disappearance of nitrates before they began to accumulate again in the Dunkirk gravelly sandy loam used by Wilson and Wilson (9), was not

enough to influence greatly the course of nitrification in Edmonton loam, which is much richer in organic matter. It should be pointed out, however, that this proportion of residues is of the same order as that added in the top six inches by the sods of our four crops in the field, where they were followed by significantly different nitrification rates (6). On the other hand, the uniformly favourable conditions for nitrification provided in the laboratory experiments would not usually be realized in the field.

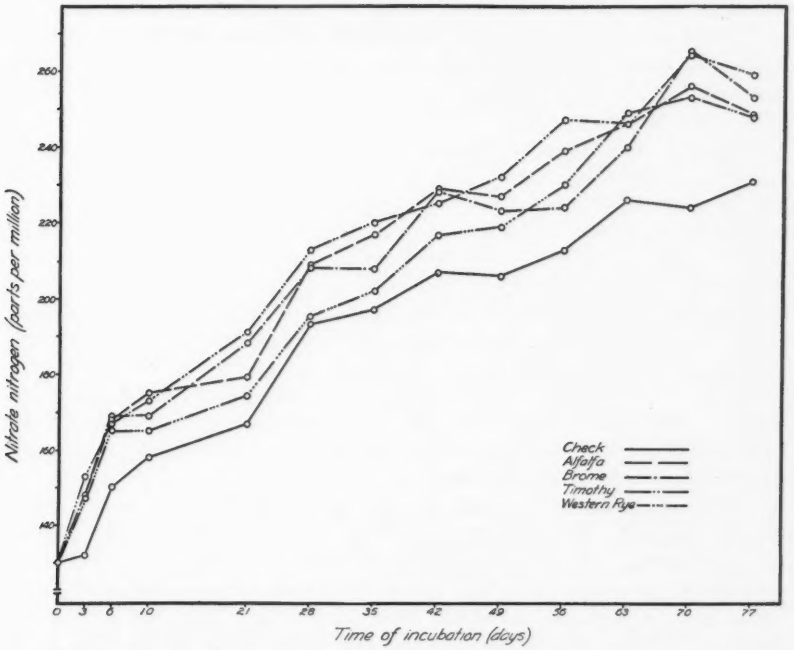

FIG. 1. *Rate of nitrification in Edmonton loam in presence of various crop residues. Series II, 1930.*

Preparation of Extracts of Fermenting Residues

For the assays with *Penicillium* and wheat plants, the residues were not used directly but in the form of extracts. Two series of experiments were carried out in which portions of the residues were fermented in a mixture with washed sand in Mason jars. In Series I they were mixed with sand in the proportion of 15% dry weight of the residues, a proportion roughly equal to that of organic matter in Edmonton loam. In Series II the same proportion of alfalfa residues was used, but the grass residues were added to their respective jars on the basis of equivalent quantities of organic matter, it having been discovered in the meantime that their ash content was abnormally high because of the impossibility of freeing the fibrous roots completely from

adhering soil particles. In both series of experiments the tops of the jars were left open for aeration, but in the first the one-quart jars were nearly filled by the quantities used, while in the second the two-quart jars substituted were only about half-filled, thus making it convenient to "cultivate" (stir up) weekly this synthetic "soil", as a precaution against the development of partially anaerobic conditions at the bottom. Five sets of jars were prepared for each experiment, representing fermentation periods of 0, 3, 6, 9, and 12 weeks. The mixture in each jar was made up to optimum moisture content, taken as 50% of the water-holding capacity, with distilled water to which had been added for each jar 10 cc. of a suspension of soil organisms made by shaking 100 gm. of Edmonton surface loam with 200 cc. of water. The jars were stored at 25° C., and weighed every two or three days for the purpose of making up to optimum moisture content.

At the end of the fermentation period, the contents of each jar were transferred to an eight-litre bottle and extracted with five parts by weight of distilled water to one part of the "soil", by shaking for 10 min. at 20° C. in a large shaking machine. The extracts were filtered and stored in Winchesters at 0° C. They were brought to a uniform nitrate content by adding calcium nitrate up to the concentration found in the one naturally richest in nitrate. The initial value (unfermented) in Series I was 2 to 3 p.p.m., but this dropped to less than 1 p.p.m. in three weeks and remained stationary thereafter, perhaps owing to the development of partially anaerobic conditions. In the better aerated Series II, nitrate increased steadily to about 60 p.p.m. at 12 weeks, and all extracts in this series were made up to this concentration. The portions used for the assays with *Penicillium* were sterilized by filtration through Pasteur-Chamberland candles.

Certain properties of these extracts which might be related to their activity were determined. The oxidation-reduction potentials did not vary significantly. Hydrogen ion concentrations fell chiefly in the range pH 7 to 8, with the higher values in the extracts of fermented, as compared with fresh, residues; there were no consistent differences between the extracts of the different residues. The remaining properties determined—total sugar, total soluble organic matter, lead acetate precipitate, and colour intensity—did show significant differences, and the data for Series II, the better aerated set of jars, are assembled in Table II.

Total sugar was determined as reducing sugar in an aliquot of 50 cc. after clarifying the extracts with lead acetate, deleading with disodium phosphate, and inverting with citric acid. Total soluble organic matter was determined by evaporating 10-cc. portions of the extracts to dryness and igniting. Relative colour intensity of the extracts after passing through a Pasteur-Chamberland filter was determined with a colorimeter, using the palest extract, that of alfalfa after 12 weeks' fermentation, as a standard. The precipitate obtained by adding 1 cc. of molar lead acetate solution to 25 cc. of extract in a test-tube, shaking, centrifuging, filtering with washing, drying *in vacuo* at 98° C., and weighing, gives another estimate of substances colouring the extracts. In the

TABLE II

PROPERTIES OF EXTRACTS OF FERMENTING CROP RESIDUES, SERIES II

Residue \ Period of ferment'n	None	3 wk.	6 wk.	9 wk.	12 wk.
Total sugar (mg. in 100 cc.)					
Alfalfa	17.2	15.0	6.6	6.6	0.0
Brome	38.6	4.0	13.8	6.6	15.0
Timothy	18.0	10.2	9.4	4.0	0.0
Western rye	59.8	23.6	6.0	18.0	21.0
Total soluble organic matter (mg. in 100 cc.)					
Alfalfa	215	57	73	73	74
Brome	119	146	32	134	106
Timothy	190	137	127	137	100
Western rye	154	57	89	110	68
Lead acetate precipitate (mg. in 100 cc.)					
Alfalfa	79	128	52	46	50
Brome	120	176	86	67	86
Timothy	113	180	99	84	115
Western rye	76	125	66	69	58
Relative colour intensity					
Alfalfa	1.5	3.3	1.7	1.0	1.0
Brome	3.2	5.5	3.2	1.9	3.1
Timothy	3.0	5.5	4.7	3.3	4.7
Western rye	1.5	3.2	1.7	1.5	1.1

Colour quality key: 1.0–1.1 pale yellow; 1.5–1.7 yellow; 1.9 deep yellow; 3.0–3.3 reddish-brown; 4.7–5.5 dark reddish-brown.

table, the analytical values are converted to the basis of 100 cc. extract for ease of comparison.

In sugar, western rye extract was highest and brome next. In all residues the sugar tended to disappear during fermentation, though not at a uniform rate. The same is roughly true of soluble organic matter; here alfalfa and timothy are highest initially. Total organic matter does not appear to be related to colour of extract, but lead acetate precipitate obviously is.

Assays with Penicillium

The blue mould *Penicillium* was selected because it is a rapid-growing organism forming a thick compact mat of mycelium which can be filtered and weighed readily. Moreover it is a typical soil-inhabiting fungus.

Three series of assays were made of the water extracts of fermenting residues. In Series I, the extracts came from the first lot of fermented mixtures, made during the early summer while the residues collected that spring were still quite fresh. Series II and III were made with extracts of the second lot, fermented after the dried, ground residues had been an additional seven months in storage. The growth of the fungus at 20° C. on Czapek's nutrient solution in 200-cc. Erlenmeyer flasks, to which extracts were added in graduated quantities, was measured by the dry weights of the mycelial mats produced. The flasks were liberally and uniformly seeded with freshly produced spores and allowed to incubate four days in Series I and II, and two days in Series III, before adding the extract, in order to avoid complicating the results by the possible effect of the extract on germination. The fungus was filtered off seven days later, experience having shown that this covered the period of maximum growth rate. The concentration of the Czapek solution was adjusted so that after the addition of the graduated quantities of extract to the flasks the final concentration of nutrients was standard in all flasks. The final volume of solution and extract was 60 cc. in all flasks, except in the first group (no fermentation) of Series I, in which the addition of 10, 20, and 30 cc. of extract brought it to 60, 70, and 80 cc. respectively. The results are given in Tables III, IV, and V.

TABLE III

MEAN WEIGHTS (MG.) OF TRIPLICATE* CULTURES OF *Penicillium* CONTAINING EXTRACTS OF FERMENTING CROP RESIDUES

Series I, 1930

Kind of residues	Volume of extract added, cc.	Length of fermentation of residues		
		None	6 wk.	12 wk.
Control	—	257	379	353
Alfalfa	10	475	530	354
	20	555	585	389
	30	626	555	381
Brome	10	464	514	364
	20	587	526	389
	30	658	538	383
Timothy	10	437	568	363
	20	584	489	383
	30	607	202	381
Western rye	10	431	524	375
	20	535	534	373
	30	668	617	365

* *Control values in Tables III, IV, and V are means of 9 replicates.*

For no fermentation time and 6 weeks:

Standard error = *83*

Necessary difference between control and treatment means = *111 to 133*†

Necessary difference between treatment means = *118 to 152*†

† *Vary owing to accidental gaps in data.*

In the first series of assays, reported in Table III, only the unfermented and the 6- and 12-week extracts were used. It will be seen that extracts of unfermented residues and of residues fermented six weeks, except the highest concentration of timothy six-week extract, stimulated the growth of *Penicillium*. Similar experiments by Newton, Lehmann, and Clarke (5) in the same laboratory had demonstrated that phenolic substances ordinarily toxic to organisms exerted in high dilution a stimulatory effect on growth. A series of graduated dosages on the fungus *Helminthosporium sativum* produced a physiological curve of stimulation and inhibition. The same mechanism seems applicable to the results with the six-week timothy extract, but in the other cases the effect seems more like that of a vitamin, since there is no tendency to inhibition of growth with increasing concentration. It has already been mentioned that in the assays of unfermented residues reported in Table III, the volumes of culture solution were increased by the addition of the extracts, and this was suspected of causing part of the increased growth. This defect in technique did not occur in the rest of the work. All effects, whether of stimulation or inhibition, had practically disappeared in the extracts from residues fermented 12 weeks.

The second series of assays was made with extracts from the second batch of the fermented residues, which differed from the first batch mainly in the weekly stirring, or aeration, of the sand-residue mixtures in the jars. This,

TABLE IV

MEAN WEIGHTS (MG.) OF TRIPLICATE CULTURES OF *Penicillium* CONTAINING EXTRACTS OF FERMENTING CROP RESIDUES

Series II, 1931

Kind of residues	Volume of extract in 60 cc.	Length of fermentation of residues				
		None	3 wk.	6 wk.	9 wk.	12 wk.
Control	—	298	394	321	347	329
Alfalfa	10	364	362	375	322	298
	20	417	441	427	331	398
	30	437	412	445	344	364
Brome	10	398	50	408	366	388
	20	465	27	432	365	418
	30	463	2	443	395	437
Timothy	10	412	44	418	364	404
	20	470	7	347	390	427
	30	485	29	337	435	463
Western rye	10	357	191	339	374	512
	20	426	227	412	360	557
	30	450	311	385	378	525

Standard error = *31*
Necessary difference between control and treatment means = *41*
Necessary difference between treatment means = *50*

of course, could not influence the character of the extract from the unfermented residues, yet it will be seen in Table IV that the vitamin-like effects were less marked. The dried, ground residues had been an additional seven months in storage, and it is known that vitamins may diminish under these conditions. The aeration may, however, have accelerated the rate of both appearance and disappearance of transitory substances, and perhaps accounts for the lack of any pronounced inhibition of growth by timothy extract at six weeks. Fortunately the three-week extracts were included in this series, and both brome and timothy showed marked toxicity at this stage, while growth in the presence of western rye extract was below that in the control and in the alfalfa. The vitamin-like effects, where they appeared, were not significantly different for the 20 and 30 cc. additions of extract. In the longer fermentation periods, such effects as persisted were practically all stimulatory in nature.

The third series of assays was done with portions of the same extracts used in Series II, but after they had been kept about a month in storage at 0° C. Certain additional refinements of technique were introduced, in the hope of reducing the variability of the results, notably the use of a single-spore culture of the test organism. The other main difference from the previous series was in the use of larger increments of extract, viz., 10, 30, and 50 cc. (as compared with 10, 20, and 30 cc.) with the object of surpassing definitely

TABLE V

MEAN WEIGHTS (MG.) OF TRIPLICATE CULTURES OF *Penicillium* CONTAINING EXTRACTS OF FERMENTING CROP RESIDUES

Series III, 1931

Kind of residues	Volume of extract in 60 cc.	Length of fermentation of residues				
		None	3 wk.	6 wk.	9 wk.	12 wk.
Control	—	320	391	403	407	382
Alfalfa	10	508	408	414	379	329
	30	583	584	503	405	404
	50	541	423	504	441	412
Brome	10	465	194	436	393	412
	30	548	181	526	437	430
	50	551	89	546	480	376
Timothy	10	531	159	439	392	395
	30	593	131	432	479	455
	50	553	12	330	513	379
Western rye	10	487	312	396	368	354
	30	571	205	474	459	430
	50	560	108	509	471	477

Standard error = *33*
Necessary difference between control and treatment means = *43*
Necessary difference between treatment means = *53*

the maximum in case the vitamin-like effect were merely a stimulation by a toxic substance in low concentration. The results suggest, however, that in most cases this effect was not due to a toxic substance. The maximum stimulation appears to have been attained by the addition of 30 cc. of extract and, comparing treatment with control values, there was no inhibition by 50 cc. of extract. The toxic effect, however, which again showed plainly, though less extremely than in Series II, in the three-week extract of the grasses, increased with the volume of extract added. There was also an appreciable inhibition of growth by 50 cc. of timothy extract from the six-week fermentation period.

A singular feature of the control cultures, to which no plant extract was added, was the complete absence in Series III of the typical green colour of the spore mass with which the mycelial mat was covered. Apparently the particular single-spore strain used in this series of cultures was incapable of developing its normal pigment in the absence of the organic supplement represented by the extracts. This did not happen in Series I or II.

Assays with Wheat Plants

Wheat plants were used as test organisms in much the same way as *Penicillium*. The wheat was grown in glazed one-gallon crocks of pure sand half-saturated with Hoagland's nutrient solution and, like the extract jars, inoculated with a suspension of soil organisms. The kernels were seeded long enough before the fermenting residues were due to be extracted, to give them time to germinate and emerge, when they were thinned to six well-spaced, uniformly vigorous plants per crock. The extracts were then added periodically in quantity sufficient to maintain optimum moisture content in the sand culture which transpired least, the necessary additional moisture in the other cultures being made up with distilled water. Nutrients were added from time to time in accordance with ordinary practice. The moisture content of the controls was maintained with distilled water to which had been added nitrate in the same concentration as in the extracts. The plants were harvested six days after the extracts were exhausted.

Two series of assays were carried out, corresponding to the two lots of fermented residues. The results of both are given in Table VI. In Series I, comparisons must be limited to weights within each fermentation period, i.e., in a single column, since the wheat cultures were set up on five successive dates at intervals of three weeks, corresponding to the end of the fermentation periods of the various lots of residues. This probably made less difference to the *Penicillium* cultures grown under controlled conditions in the laboratory*, but did result in exposing the different lots of wheat plants to markedly different environments in the greenhouse as the season progressed from June to August, 1930. In Series II the wheat plants were all

* *Even here significant differences occur between control values, including those of Penicillium Series II and III, each of which was set up at five successive two-day intervals. Difficulty in duplicating conditions of inoculation on different days is a common experience of mycologists.*

TABLE VI

TOTAL DRY WEIGHT (GM.) OF SIX WHEAT PLANTS GROWN IN SAND CULTURE WATERED WITH EXTRACTS OF FERMENTING CROP RESIDUES

Kind of residues	Length of fermentation of residues				
	None	3 weeks	6 weeks	9 weeks	12 weeks
Series I					
Control	26.9	16.2	14.5	9.0	8.8
Alfalfa	11.7	11.6	3.1	8.3	8.6
Brome	32.0	22.4	4.4	5.7	10.3
Timothy	21.5	21.5	3.6	6.7	10.2
Western rye	26.8	24.7	7.4	7.4	8.8
Series II					
Control	20.4	24.6	26.1	25.3	23.2
Alfalfa	27.9	23.3	34.7	34.1	26.1
Brome	36.5	29.6	30.7	32.0	25.0
Timothy	37.3	26.7	33.2	32.6	21.4
Western rye	31.6	28.3	39.1	31.7	24.1

grown at one time, the residues having been set to ferment at three-week intervals so that all completed their respective periods approximately together. This wheat series was grown during the period January to March, 1931, when it was possible to control greenhouse conditions more satisfactorily.

In Series I it was surprising to find the extracts of unfermented and three-week alfalfa exercising a depressive influence on growth, since alfalfa extract had stimulated the growth of *Penicillium* at least as much as had that of the grasses, and unlike the grasses had in no case shown toxicity to that organism. In a biological study of the decomposing sods in the field (4) it had been observed that alfalfa sod favoured the development of fungi, especially *Mucor*, to a much greater extent than did the grasses. This was suspected to be the possible cause of the unthriftiness in the wheat grown in the sand cultures. Water suspensions from the sand cultures plated out did indeed reveal a predominance of large *Mucor* colonies in those treated with alfalfa extracts. The repressive effect of the alfalfa was possibly an indirect one, exerted through its stimulation of micro-organisms which competed with or injured the roots of the wheat. Thus the prime object of the technique developed for assaying these residues, namely, to separate the effects of chemical and biological factors, was scarcely fulfilled by the wheat cultures, and from this point of view they were less successful than the *Penicillium* cultures. The figures suggest that all the extracts from residues fermented six weeks may have slightly repressed growth, but the error of the experiment is too great to permit conclusions. Certainly there is no evidence of any effect at 9 and 12 weeks.

In Series II, done with extracts of residues fermented under better conditions of aeration, there is no evidence of growth inhibition, but the unfermented and three-week alfalfa stimulated the growth of wheat less than did the other extracts, again possibly owing to the greater profusion of certain fungi in the alfalfa cultures. At 6 weeks and 9 weeks, all the extracts stimulated the growth of wheat, but there was no effect at 12 weeks.

The greater weights of the plants in Series II is partly due to the greater age of the plants at harvesting. Because of the lower rate of transpiration during the winter, they were in full flower before the supply of extracts was exhausted, whereas in Series I they were barely heading when harvested. The possibility of toxic decomposition products affecting the results with the six-week extract in Series I cannot, however, be ruled out, in view of the observation mentioned earlier, that the conditions of fermentation had inhibited nitrification.

The nitrogen content of tops and roots separately was determined in the wheat plants of both series. The data are omitted to save space, but it may be said that the chief trend discernible was towards a higher percentage in the heavier, thriftier plants.

The plating of water suspensions of the sand cultures was carried out more completely in Series II, in view of observations in Series I referred to above. Three borings were taken from each crock, just prior to harvesting. For fungi, dilutions of 1 : 1000 were used; for bacteria, 1 : 1,000,000 and 1 : 10,000,000. Media used were No. 4 and No. 18, Fred and Waksman (1) for bacteria and fungi respectively. Plates were poured in quadruplicate. The counts are given in Table VII. The wide variations in numbers do not appear to be related to the yields of wheat.

It will be remembered that all sand cultures, both those producing the residue extracts and those in which the wheat was grown, had been inoculated with a suspension of Edmonton surface loam in order to promote normal microbial balance. No wide differences were found in the kinds of bacteria on the plates, and no differences were apparent in the size of bacterial colonies from the various sand cultures. The fungi, on the other hand, varied widely in kinds and size of colonies. The control and alfalfa plates from the crocks receiving extracts of unfermented residues had chiefly large *Mucor* colonies. Brome and timothy plates had a mixed flora including a few large colonies of *Mucor*. Western rye plates had a large number of *Penicillium* colonies, with very few other forms present.

The three-week plates showed a mixed fungus flora, including *Mucor*, *Penicillium*, *Aspergillus*, and *Fusarium*. The six-week plates gave a mixed flora in the control and western rye, but scarecly any *Mucor* colonies in alfalfa, brome, or timothy. The 9- and 12-week plates likewise gave rise to a mixed flora. On the whole the plates from the unfermented residues, the 9- and 12-week fermentation periods all showed about equal vigour of fungus growth, as judged by size of colonies, and more than shown by the three- and six-week periods. The fungi on the alfalfa and brome plates in the six-

TABLE VII

NUMBERS OF BACTERIA AND FUNGI IN SAND CULTURES OF WHEAT PLANTS WATERED WITH EXTRACTS OF FERMENTING CROP RESIDUES

Series II, 1931

Residue	Fungi	Bacteria
	Thousands per gm. dry sand	Millions per gm. dry sand
No fermentation		
Control	13.1	18.0
Alfalfa	15.6	37.5
Brome	22.7	61.6
Timothy	7.8	2.3
Western rye	113.5	60.2
3 weeks' fermentation		
Control	30.8	34.1
Alfalfa	6.6	12.4
Brome	24.2	36.6
Timothy	23.0	23.7
Western rye	12.9	27.4
6 weeks' fermentation		
Control	28.8	41.6
Alfalfa	15.9	49.9
Brome	3.9	41.8
Timothy	12.8	21.9
Western rye	26.1	59.2
9 weeks' fermentation		
Control	5.6	28.1
Alfalfa	5.3	41.2
Brome	21.1	24.8
Timothy	10.0	40.7
Western rye	15.7	55.0
12 weeks' fermentation		
Control	8.3	28.6
Alfalfa	7.5	21.8
Brome	13.0	39.0
Timothy	5.3	49.5
Western rye	7.0	52.1

week group were particularly lacking in vigour. This specificity, both as to kind and vigour of colonies, is additional evidence of the biological effects of the extracts.

At the time of taking samples of the sand cultures for plate counts, samples were also secured for the determination of pH. This ranged from 7.2 to 8.2. Cultures watered with fresh and three-week alfalfa extracts were a little lower than the others, but all were within the range of healthy soil.

Discussion

While current soil theory for the most part rejects toxic decomposition products as factors in crop sequence effects in normally aerated soils, such an extreme view seems not wholly tenable in the light both of our own results and of certain other cases reported in the literature. We did not indeed demonstrate with certainty any toxicity in the wheat plant cultures. The extracts used in Series I were from poorly aerated jars, possibly comparable to "sour" soils. The effect of the alfalfa extract was thought to be indirect. But the effect of the extracts, even from well aerated jars, on the growth of *Penicillium* was undoubted, and all the extracts showed characteristic effects on the fungus flora of the sand cultures. This indicates the likelihood that decomposition products of crop residues influence soil micro-organisms, whose activities influence in turn the productivity of the soil and may also affect the growth of plants by parasitism.

Both the toxic and vitamin-like effects observed deserve equal consideration, since in practice it does not matter whether the differences it is desired to explain in yield and composition of wheat following various other crops on similar soil arise in particular cases from stimulation or inhibition of soil microbial activity. Further, it need not be assumed that only organic decomposition products are active in this respect. The vitamin-like effect may be due to "minor" or "trace" elements absent from our culture solutions and present in the extracts. Again, some species of plants accumulate toxic elements (e.g., selenium in *Astragalus*) which, on decomposition of the residues, must be liberated in the soil.

While due caution must be observed in applying laboratory results to field conditions, there is no doubt that crop residues do cause differences in the microbiological activity and yielding power of soil, and we have only to consider whether these experiments suggest a reasonable explanation. There is no need to discount the importance of other factors, such as differences in the C : N ratio of the residues, but our results do suggest that the biological action of substances leaching out of crop residues in the soil also play a part in modifying its productivity.

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AGRICULTURAL METEOROLOGY: A STATISTICAL STUDY OF CONSERVATION OF PRECIPITATION BY SUMMER FALLOWED SOIL TANKS AT SWIFT CURRENT, SASKATCHEWAN[1]

By J. W. Hopkins[2]

Abstract

Changes in the moisture content of summer fallowed soil in tanks 15 in. in diameter and 5 ft. deep set in pits in the centre of field plots at the Dominion Experimental Station, Swift Current, Saskatchewan, which have been recorded at fairly frequent intervals during the summer months since 1922, provided two series of data, comprising observations in May and June and those in July and August. These have been analysed statistically in order to relate conservation to precipitation after making allowance for other variable factors. Five-factor regression equations, fitted by the method of Least Squares, gave multiple correlation coefficients of 0.95 and 0.96 between the computed and observed conservation in the two series. It is inferred from these equations that under average conditions a one-day rain to the amount of 0.36 in. in May-June and 0.46 in. in July-August would be required just to offset the subsequent evaporation during a 10-day period. Increased penetration and retention of the heavier rainfalls is very definitely indicated, and it is calculated that whereas on the average 66% of a one-day rain of 1 in. in May-June would still be conserved at the end of 10 days, only 30% would be retained if the same amount was received in five daily showers each of 0.20 in. The intensity as well as the total amount of precipitation is thus emphasized as a factor of prime importance in its relation to soil moisture under semi-arid conditions.

Introduction

In regions of limited rainfall, the conservation by the soil of such amounts of precipitation as are received is a factor of the first importance in the conduct of agricultural operations. This is the case in the Prairie Provinces of Canada, where an appreciable proportion (according to Taggart (8) about eight million acres in Saskatchewan alone) of the cultivated land is summer fallowed each year in order to increase the supply of moisture available for the succeeding season's crop.

The amount of moisture thus accumulated is subject to considerable fluctuation, for the summer precipitation at any specified point in this region varies widely from year to year not only in total amount, but also in its mode of occurrence. Even in favourable seasons, a considerable aggregate is received in the form of daily amounts of 0.30 in. or less (4), whilst in months of below-average rain, the entire amount recorded may fall in this way. As a certain amount of the moisture received at any time as rain does not penetrate deeply into the soil, but is retained by the surface layer and subsequently evaporated directly into the atmosphere, it can readily be foreseen that the efficiency of conservation by the soil of different intensities of rain

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will not be the same, and that three separate showers of 0.25 in. for example, cannot be assumed to be equivalent in effect to one fall of 0.75 in. Thus it was the opinion of Barnes (1) that under western Canadian conditions the moisture from showers of 0.25 in. or less is generally lost within a day or two by evaporation. Daniel and Harper (2) adopted 0.50 in. per day as the lower limit of effective rainfall in Oklahoma, and a figure of 0.50 in. was also arrived at by McLaughlin, Blaney, and Taylor (7) in considering the disposition of winter rainfall in the Santa Ana river area of California. These last authors further observe that "every rainfall period must be studied separately in order to arrive at the penetration for the season, since the same seasonal precipitation gives entirely different amounts of penetration under different conditions of intensity and distribution."

As far as the writer knows, the actual penetration into the soil of individual rainfalls has not been studied quantitatively in any great detail in Canada. The technical difficulty of obtaining sufficiently accurate data for this purpose by the usual soil-boring procedures is considerable, if not insurmountable; whilst the exceedingly imperfect correlation between the rate of loss of water vapour from a body of soil, and that observed from instruments such as atmometers or from tanks or pans presenting a free-water surface, has been emphasized by several writers, of whom Thornthwaite and Holzman (9) may perhaps be noted as the most recent. It seemed therefore that advantage should be taken of the information relevant to this subject accruing from the operation of the deep, water-tight soil tanks at the Soil Research Laboratory, Dominion Experimental Station, Swift Current, Saskatchewan. This information is probably unique in that the tanks have been under continuous observation since the year 1922. The following sections of this paper accordingly describe the results of a statistical examination of the accumulated records, which were made available to the author through the co-operation of Dr. J. L. Doughty of the Soil Research Laboratory.

Observational Data

The construction, location, and management of the tanks has been described by the late S. Barnes (1). It will therefore suffice to mention here that they are made of galvanized iron, and are circular in cross-section, 15 in. in diameter and 5 ft. deep. The soil with which they are filled is said to have been uniform and well mixed, and to have been placed in the tanks in layers corresponding to the natural soil horizons. The tanks are set in pits, so that the top of the soil in each tank is approximately at ground level. A regular rotation is imposed, and in any particular season some of the tanks grow crops of wheat, grasses, etc., whilst others are summer fallowed. In the present communication, attention is confined to the latter.

For these, the observations consisted of the weights, determined at more or less regular intervals during the summer months, of the replicate tanks (usually four or six in number) fallowed in each of the years 1922 to 1938 (excluding 1923), together with daily records of precipitation secured from a

TABLE I

CONSERVATION OF PRECIPITATION BY SUMMER FALLOWED SOIL TANKS, SWIFT CURRENT, 1922–1938

May and June					July and August			
Period		No. of days	Precipitation, in.	Conservation, in.	Period		No. of days	Precipitation, in.	Conservation, in.
1 June to	15 June/22	14	0.37	−0.31	15 June to	19 July/22	34	5.13	+2.38
16 May	30 May/24	14	.89	+ .27	19 July	1 Aug./22	13	.20	− .38
30 May	6 June/24	7	.15	− .09	1 Aug.	12 Aug./22	11	1.15	+ .55
6 June	23 June/24	17	1.85	+ .71	12 Aug.	21 Aug./22	9	1.10	+ .33
23 June	4 July/24	11	.51	− .09	4 July	12 July/24	8	.67	+ .14
12 May	26 May/25	14	.28	− .14	12 July	18 July/24	6	.21	+ .11
26 May	9 June/25	14	1.21	+ .50	18 July	24 July/24	6	.37	.00
9 June	15 June/25	6	.59	+ .06	24 July	1 Aug./24	8	.81	+ .09
15 June	23 June/25	8	.00	− .19	1 Aug.	6 Aug./24	5	1.13	+ .67
23 June	29 June/25	6	.01	+ .11	6 Aug.	11 Aug./24	5	.73	+ .50
12 May	14 June/26	33	1.97	+ .22	11 Aug.	18 Aug./24	7	.15	− .20
14 June	2 July/26	18	1.67	+ .30	18 Aug.	25 Aug./24	7	.27	+ .16
16 May	14 June/27	29	4.75	+3.75	29 June	6 July/25	7	.18	− .24
14 June	25 June/27	11	.45	− .08	6 July	13 July/25	7	.66	+ .09
10 May	1 June/28	22	.52	− .20	13 July	25 July/25	12	.10	− .16
1 June	9 June/28	8	.42	+ .22	25 July	31 July/25	6	.64	+ .36
9 June	26 June/28	17	3.33	+2.40	2 July	9 July/26	7	.47	+ .03
26 June	5 July/28	9	1.44	+1.08	9 July	16 July/26	7	1.76	+ .97
7 May	20 May/29	13	.30	+ .24	16 July	21 July/26	5	.00	− .24
20 May	4 June/29	15	1.52	+1.02	21 July	28 July/26	7	.00	− .14
4 June	10 June/29	6	.00	− .09	28 July	2 Aug./26	5	.00	− .02
10 June	21 June/29	11	2.18	+1.58	2 Aug.	23 Aug./26	21	2.77	+1.44
21 June	28 June/29	7	.40	− .19	25 June	8 July/27	13	1.92	+1.07
5 May	13 May/30	8	.18	+ .05	8 July	18 July/27	10	.60	+ .03
13 May	20 May/30	7	.00	− .24	18 July	25 July/27	7	.35	− .14
20 May	27 May/30	7	.15	+ .09	25 July	1 Aug./27	7	.22	− .03
27 May	2 June/30	6	.00	+ .11	1 Aug.	8 Aug./27	7	.01	− .11
2 June	10 June/30	8	.29	− .08	8 Aug.	16 Aug./27	8	1.67	+1.08
10 June	17 June/30	7	1.52	+1.35	16 Aug.	22 Aug./27	6	.43	− .05
17 June	24 June/30	7	.04	− .08	22 Aug.	31 Aug./27	9	.71	− .03
24 June	3 July/30	9	2.04	+1.49	5 July	16 July/28	11	.19	− .52
16 May	28 May/31	12	.50	+ .28	16 July	23 July/28	7	.50	− .08
28 May	4 June/31	7	.02	− .16	23 July	30 July/28	7	.08	+ .11
4 June	11 June/31	7	.02	.00	30 July	7 Aug./28	8	1.06	+ .55
11 June	19 June/31	8	.27	− .02	7 Aug.	13 Aug./28	6	.00	− .17
19 June	25 June/31	6	.14	.00	13 Aug.	20 Aug./28	7	.14	− .11
25 June	2 July/31	7	1.24	+ .60	28 June	9 July/29	11	.53	+ .02
9 May	25 May/32	16	.55	+ .06	9 July	16 July/29	7	.77	+ .50
25 May	1 June/32	7	.21	+ .09	16 July	23 July/29	7	.00	− .42
1 June	15 June/32	14	1.92	+ .77	23 July	29 July/29	6	.00	− .14
					29 July	6 Aug./30	8	.00	− .02
15 June	22 June/32	7	.00	+ .03	3 July	8 July/30	5	.13	− .02
22 June	29 June/32	7	.39	+ .05	8 July	17 July/30	9	.02	− .56
11 May	31 May/33	20	1.26	+ .56	17 July	22 July/30	5	.27	− .02
31 May	8 June/33	8	.28	− .02	22 July	29 July/30	7	.17	− .03
8 June	15 June/33	7	.21	− .06	2 July	9 July/31	7	.41	+ .08
23 May	14 June/34	22	2.70	+1.69	9 July	16 July/31	7	.26	− .19
14 June	21 June/34	7	1.01	+ .06	16 July	22 July/31	6	.07	− .38
21 June	3 July/34	12	1.00	+ .16	22 July	12 Aug./31	21	1.96	+1.24
16 May	28 May/35	12	.33	+ .09	29 June	11 July/32	12	3.21	+2.65
28 May	6 June/35	9	.15	.00	11 July	20 July/32	9	.62	+ .05
6 June	18 June/35	12	.86	+ .33	20 July	27 July/32	7	.37	− .35
18 June	26 June/35	8	1.36	+ .78	27 July	3 Aug./32	7	.62	+ .39
26 June	3 July/35	7	1.06	+ .47	15 June	17 July/33	32	2.48	+ .64
3 June	23 June/36	20	1.71	+ .75	3 July	11 July/34	8	.16	− .20

TABLE I—*Concluded*

CONSERVATION OF PRECIPITATION BY SUMMER FALLOWED SOIL TANKS, SWIFT CURRENT, 1922–1938—*Concluded*

May and June					July and August				
Period		No. of days	Precipitation, in.	Conservation, in.	Period		No. of days	Precipitation, in.	Conservation, in.
23 June	6 July/36	13	.38	− .19	11 July	19 July/34	8	.15	+ .02
19 May	2 June/37	14	.71	+ .52	19 July	26 July/34	7	.87	− .42
2 June	10 June/37	8	.12	− .13	3 July	10 July/35	7	1.12	+ .41
10 June	16 June/37	6	.17	− .14	10 July	17 July/35	7	.00	− .31
16 June	25 June/37	9	.34	− .22	17 July	24 July/35	7	.56	+ .03
9 May	26 May/38	17	.36	.00	6 July	14 July/36	8	.20	− .14
26 May	1 June/38	6	.23	− .46	14 July	22 July/36	8	.35	− .05
1 June	7 June/38	6	.14	− .14	25 June	7 July/37	12	.05	− .25
7 June	14 June/38	7	.97	+ .55	7 July	21 July/37	14	.98	+ .49
14 June	22 June/38	8	.59	+ .05	21 July	11 Aug./37	21	.55	− .27
22 June	28 June/38	6	.18	− .19	28 June	5 July/38	7	.08	− .27
					5 July	13 July/38	8	.19	− .17
					13 July	19 July/38	6	.04	+ .06
					19 July	26 July/38	7	.37	− .08
					26 July	4 Aug./38	9	.17	− .14
Average		10.9	.78	+ .31	Average		9.0	.63	+ .15

rain-gauge adjacent to the tanks. Given the diameter of the tanks (15 in.), and the fact that 1 lb. of water at 15° C. occupies a volume of 27.73 cu. in., it follows that each change in weight of 1 lb. per tank represents a gain or loss of moisture equivalent to $27.73 \div \pi(7.5)^2$ or 0.157 in. in depth. By means of this relation the changes in weight over the various periods of record have been expressed in terms of inches of moisture, and are shown in Table I together with the corresponding totals of precipitation.

Results

Regression Analysis

From a priori considerations it may be inferred that the efficiency of conservation should show progressive changes correlated with the seasonal trends of soil and air temperature and wind velocity, which in a continental climate are quite pronounced. The available data are not particularly well adapted to the investigation of this point in detail, but are sufficiently numerous to permit of subdivision into those recorded during the first half of the season, namely May and June, and those falling in July and August. This is at once indicative of some seasonal effect, for although the average number of days between weighings (and hence the exposure to evaporation), shown at the foot of Table I, was greater in May-June than in July-August, the ratio of mean conservation to mean precipitation, at 0.31 : 0.78 in., or 40%, is more favourable in the former than in the latter period, for which it is 0.15 : 0.63, or 24%.

Fig. 1 shows the relation between the total precipitation and conservation observed in the individual periods. The seasonal shift is manifested in the tendency of the solid points representing the observations during July-August to lie below those for May-June. It may also be noted that the maximum total precipitation recorded in any period during which there was no net conservation of moisture is 0.87 in. for July-August, as compared to 0.52 in. for May-June.

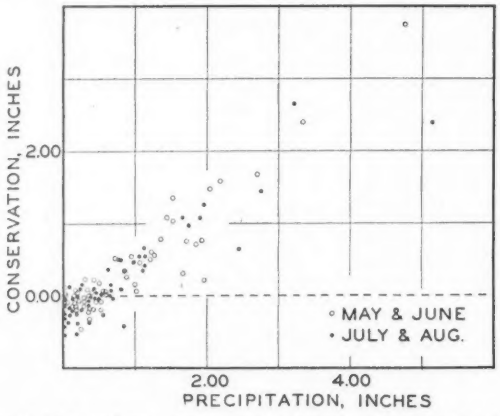

FIG. 1. *Precipitation and net conservation of moisture by soil tanks observed at Swift Current, Sask., 1922-1938. Observational periods of variable length. (See Table 1).*

The impression gained from Fig. 1 is that on the average there was an approximately linear relation between precipitation and conservation, but that the results for individual periods may differ rather widely from the general trend. This trend is specified for the May-June and July-August observations respectively by the regression equations, fitted to the data by the method of Least Squares:

$$c = -0.25 + 0.72\,p$$

and

$$c = -0.24 + 0.61\,p \qquad \text{(I)}$$

in which c denotes conservation, expressed as inches of depth, and p precipitation, likewise in inches.

These, however, can be regarded only as first approximations, their most obvious defect being that they do not take into account the differences in duration of the individual observation periods, and hence in the amount of evaporation to be anticipated. Inclusion of this factor significantly improved the regression equations, which now became as a second approximation:

$$c = -0.03 + 0.84\,p - 0.029\,d \quad \text{(May-June)}$$

and

$$c = -0.03 + 0.76\,p - 0.039\,d \quad \text{(July-August)} \qquad \text{(II)}$$

d being the interval between weighings in days.

Under similar atmospheric conditions, evaporation will be greater when the upper layers of soil are moist than when they are dry. The amount of evaporation during some of the observational periods may therefore have been influenced by precipitation occurring before, as well as during, the period in question. The total precipitation, as inches of depth *(a)* recorded during the seven days antecedent to each observational period was accordingly introduced into the two regression equations as an additional variable. In both cases negative regression coefficients resulted, indicating that in general above-average antecedent precipitation was associated with increased subsequent evaporation. However, only the coefficient for May-June can be regarded as statistically significant, leading to the conclusion that the observations in the latter part of the season were not subject to any considerable variation from this cause.

After the introduction of the quantity a the regression equations were:

$$c = +0.03 - 0.13a + 0.84\,p - 0.030\,d \quad \text{(May-June)}$$

and

$$c = +0.01 - 0.05a + 0.76\,p - 0.035\,d \quad \text{(July-August).} \qquad \text{(III)}$$

The effect of precipitation in moistening the upper soil may clearly be operative during observational periods as well as preceding them. It might perhaps be expected therefore that, other things being equal, the total loss of moisture recorded would be greater when rain occurred early in an observational period than when a similar amount fell towards the end, the first days being dry. In order to investigate this possibility quantitatively, a time factor was introduced into the regression function, consisting of the sum of products *(t)* of the daily rainfalls recorded multiplied by the ordinal numbers (reckoned from the end of each period) of the days on which they occurred. This was found to account for a significant fraction of the residual variance of conservation in both May-June and July-August. The Least Squares solution for the regression equations now took the following form:

$$c = +0.09 - 0.12\,a + 0.74\,p - 0.034\,d + 0.0091\,t \quad \text{(May-June)}$$

and

$$c = -0.09 - 0.05\,a + 0.84\,p - 0.026\,d - 0.0074\,t \text{ (July-August).} \qquad \text{(IV)}$$

Although both of the additional regression coefficients are statistically significant, only one of them is negative in sign as anticipated. In scrutinizing the data for explanations of this discrepancy between the two series of observations, it was noted that several exceptionally heavy rains in May and June had chanced to occur early in the observational periods in which they fell. Attention was thus directed to the possibility that the relation between precipitation and conservation was not entirely linear, and that an increased effectiveness of these heavy rains was being confounded with the time factor. This point was investigated by expanding the regression function to include a quadratic term in precipitation, namely the sum of the squares of the daily amounts of rain recorded in each period. The sum of the squares, rather than the square of the sum, was used in order to discriminate between heavy

daily rains and similar totals received in smaller amounts over several days. Both series of observations yielded quadratic regression coefficients significant beyond the 1% point and positive in sign, indicating a proportionately greater conservation of the more intense rains. In view of this result, the cubic term in precipitation was also fitted, but proved to be statistically negligible. After the inclusion of the quadratic term, the regression equations were:

$$c = +0.08 - 0.12\,a + 0.34\,p + 0.42\,p^2 - 0.024\,d + 0.0120\,t \quad \text{(May-June)}$$

and

$$c = -0.07 - 0.03\,a + 0.52\,p + 0.33\,p^2 - 0.022\,d - 0.0000\,t \quad \text{(July-August).} \quad \text{(V)}$$

The time factor within periods, t, is now reduced to insignificance in the July-August series, but its coefficient is still positive in the May-June equation, implying a greater conservation (temporarily at least) of precipitation received in the earlier part of the observational periods. No ready explanation of this tendency presents itself, except possibly the reduction in both soil and air temperatures following rainstorms, which might be expected to be more pronounced, and to persist longer, in the first half of the season. In the absence of soil temperature records, this point cannot be explicitly resolved.

The effect of variations in air temperature was however examined by the inclusion of this factor in the observational series. It was judged that evaporation would be affected, if at all, to a much greater degree by the daily maximum than by the mean air temperature, and the former quantity was accordingly the one employed. Even so, the resulting improvement in the goodness of fit of both the May-June and July-August regression equations was statistically quite insignificant. It must be concluded, therefore, that within series the recorded variations in daily maximum air temperature, apart from those associated with the incidence of precipitation, were without demonstrable effect on the amounts of moisture conserved by the soil tanks.

Adequacy of Regression Function

After the fitting of Equations (V), an analysis of the variance of the individual observations of moisture conservation gave the following results:

Variance attributable to	May-June		July-August	
	D.f.	Mean Square	D.f.	Mean Square
Linear effect of precipitation	1	26.3187**	1	19.0990**
Additional effect, length of obs. period	1	.9752**	1	1.0610**
Additional effect, antecedent pptn.	1	.2126*	1	.0544
Additional effect, time factor within periods	1	.2646*	1	.2525*
Additional quadratic effect of pptn.	1	.3562**	1	.2838**
Residual	59	.0427	64	.0358

**Exceeds mean square residual, 5% level of significance.*
***Exceeds mean square residual, 1% level of significance.*

The mean square residual is of the same order in both series of observations. Although this is small in comparison with the components accounted for by the various terms of the regression function, it nevertheless corresponds to a standard deviation of conservation of about 0.2 in. per observational period, which as noted previously had an average length of 11 days in May-June and 9 days in July-August. It is therefore desirable to ascertain whether the above mean square residuals are of the magnitude to be expected from the errors inherent in the data alone, or whether they indicate that factors exerting a sensible effect upon evaporation or conservation have still not been taken into account.

Errors in the data may arise from a variety of causes, of which the following may be mentioned specifically. (i) Differences in the change in weight recorded for replicate tanks during the same period of observation, whether due to actual variations in moisture retention, or simply to errors in weighing. (ii) Discrepancies between periods arising from the fact that the hour of weighing the tanks did not coincide with that of reading the rain-gauge. These could only occur when rain fell between the reading of the gauge and the weighing of the tanks, and in view of the fact that in most instances no precipitation whatsoever was recorded on the day on which the weighing took place, they could not have been a major source of error. It is almost certain, however, that some slight irregularities were introduced in this way. (iii) Possible discrepancies in weight between periods, arising from losses of surface soil through wind action. It might be thought that these would be included in (i) above, but this would be the case only in so far as such occurrences affected replicate soil tanks differentially.

Quantitative information is available respecting only errors of type (i). An analysis of the variance of the individual changes in tank weights for 16 periods selected at random from the total number available led to the deduction of a mean square error, based on 72 degrees of freedom, of 0.04437 per tank per period, in terms of inches of moisture. The mean square error of an average of four tanks (the minimum number observed in any period) would thus be 0.01110, i.e., about one-quarter of the mean square residuals shown on page 394 above. Even after allowing for the probable additional effect of random errors arising from sources (ii) and (iii) therefore, it seems likely that the specification of moisture conservation by the regression formulae is still susceptible of some refinement. Such a conclusion is readily reconcilable with the fact that whereas seasonal effects have been recognized in the present analysis only by the subdivision of the data into two arbitrary categories, in actuality seasonal changes, such as those in soil temperature, proceed in a continuous and gradual manner. Even with this limitation, however, Equations (V) yield multiple correlation coefficients of $R = 0.96$ and 0.95 with the observed conservation, a result which must be regarded as at least moderately satisfactory.

Seasonal Trend

The statistical significance of the seasonal factors affecting the relation between precipitation and conservation is readily demonstrable by the following analysis of variance of conservation, performed on the combined series of observations.

Variance due to	D.f.	Mean sq.	F.
Average regression function	5	6.5155	166.64***
Departure of mean conservation for May-June and July-Aug. respectively from average regression function	1	16.8239	430.28***
Differences between corresponding coefficients in separate regression functions for May-June and July-Aug.	5	.0702	1.80
Residual deviations of individual observations from separate regression functions	123	.0391	

*** *Exceeds mean sq. residual, 0.1% level of significance.*

The discrepancy is for the most part attributable to the reduced average conservation of similar amounts of rain in July-August. Apart from this, the aggregate differences between corresponding regression coefficients yield a variance ratio (3) of only 1.80. This attains approximately the 10% level of significance, and is thus perhaps suggestive of some slight degree of divergence, but does not permit its reality to be asserted with any degree of confidence.

It is likely that the departure of the conservation data for May-June and for July-August from the same regression plane is due to the effect of the seasonal trend of soil temperature on evaporation. However, even had there been no modification of the regression function, the ratio of conservation to precipitation would still have been somewhat less favourable in the latter half of the season owing to the less frequent occurrence of heavy rains which, as will be seen below, are most efficiently conserved. Table II shows a statistical classification of the individual daily rainfalls recorded during the period covered by the tank observations, from which it is to be noted that whereas 40% of the total precipitation for May-June was received in daily amounts exceeding 0.75 in., only 27% of the total for July-August fell in rains of this intensity. The preponderance of extremely light showers, common to both divisions of the season, is a disadvantageous characteristic of precipitation in the western plains region, commented upon in previous publications (4, 5), that serves to explain why, over a number of years, a "surprisingly small proportion", viz., about 30% (1) of the total precipitation was found to be actually conserved during the summer fallow period.

TABLE II

CLASSIFICATION OF DAILY AMOUNTS OF PRECIPITATION RECORDED

Period	Statistic	0.01 to 0.25 in.	0.26 to 0.50 in.	0.51 to 0.75 in.	0.76 to 1.00 in.	Over 1.00 in.
May and June	Actual frequency of occurrence	226	35	12	17	10
	Percentage of total number of days with rain	75	12	4	6	3
	Aggregate inches of rain accruing	20.32	12.20	7.69	14.99	12.48
	Percentage of total precipitation	30	18	12	22	18
July and August	Actual frequency of occurrence	233	37	22	8	7
	Percentage of total number of days with rain	76	12	7	3	2
	Aggregate inches of rain accruing	15.39	13.07	13.44	6.60	8.82
	Percentage of total precipitation	27	23	23	12	15

Inferences from Regression Function

The regression functions relating the recorded changes in soil moisture to the various observed factors enable the estimated conservation of various amounts of precipitation under any arbitrarily selected set of standard conditions to be computed. As an example, Fig. 2 shows the calculated conservation of soil moisture 10 days after the receipt of varying amounts of precipitation. It is assumed that this precipitation all occurred on the first day, and that the remainder of the period was rainless. The further assumption is made that a total of 0.50 in. of rain fell during the preceding seven days. In Equations (V), therefore, $a = 0.50$ and $d = 10$. The consistently superior retention of corresponding amounts of rainfall in the first half of the season is clearly evident from the figure. The difference in favour of May-June in this respect would have been even more pronounced had the preceding week been assumed rainless, so that the surface soil was dry at the beginning of the 10-day period. However, the value of 0.50 in. adopted for a is more nearly in accordance with the average of the quantities actually recorded. Differences in slope of the two conservation curves are slight, and as noted above, the significance of these is doubtful. In both cases the curvature is positive, indicating the retention of an increasing proportion of the larger rains, which may be assumed to penetrate to a position in the soil below the zone of most active evaporation (10).

In the absence of any precipitation at all, it is calculated that on the average 0.22 in. of moisture would be lost from the soil in May-June, and 0.31 in. in July-August. As soon as the surface soil is moistened by rainfall, however, evaporation increases, so that rain to the calculated amount of 0.36 in. in May-June and 0.46 in. in July-August would be required on the first day in order just to offset the subsequent evaporation during the 10-day period.

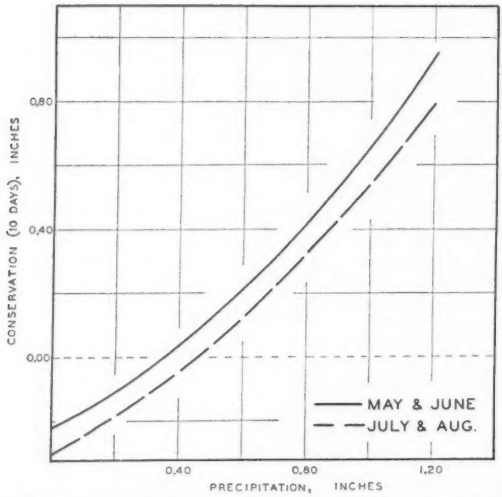

FIG. 2. *Calculated net conservation of moisture by soil 10 days after the occurrence of various one-day amounts of precipitation.*

In a sense these amounts may be regarded as the "minimum effective precipitation", although they have of course no absolute meaning apart from the specified conditions underlying their computation. Reducing the period under consideration from 10 to 5 days, for example, leads to calculated "effective minima" of 0.20 and 0.26 in. respectively, instead of the foregoing 0.36 and 0.46 in.

Fig. 3 shows the calculated percentage conservation, after 10 days, of precipitation in excess of the "effective minimum", under the conditions outlined above, and indicates the increased efficiency of the heavier rainfalls.

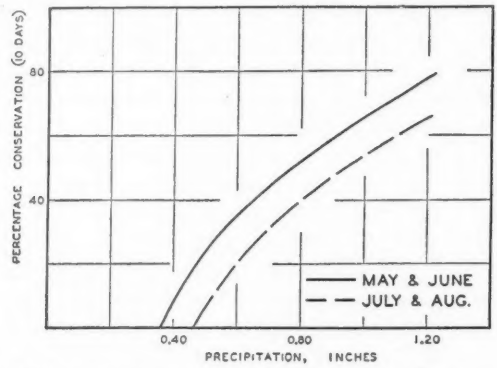

FIG. 3. *Calculated percentage conservation by soil, 10 days after occurrence, of various amounts of precipitation in excess of the one-day "effective minimum".*

This point is further illustrated in Fig. 4 which shows the computed amounts conserved at the end of 10 days (again under the same conditions as before) when 1 in. of rain is received in a single amount on the first day, in two amounts of 0.50 in. on each of the first and second days, in three amounts of 0.33 in. each on the first, second, and third days, and so on. The increased penetration and subsequent retention of the heavier rainfalls is strikingly evident. In May-June for example, 66% of a one-day rain of 1 in. is still conserved at the end of 10 days, whereas only 30% would be retained if the same amount of rain was distributed over five days. The intensity, as well as the total amount, of precipitation is thus again emphasized as a factor of prime importance in its relation to soil moisture under semi-arid conditions.

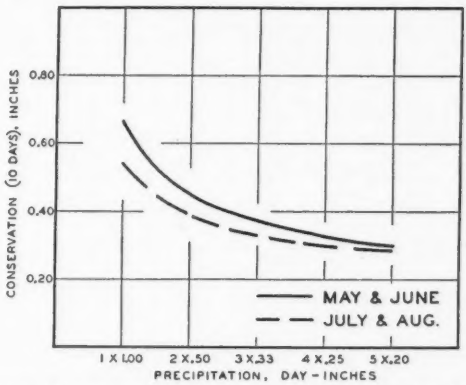

FIG. 4. *Calculated effect of intensity of precipitation on conservation by soil of moisture from one inch of rain.*

It will be realized that the results shown in Figs. 2, 3, and 4 were all obtained using a single set of values for the factors a, d, and t in the regression functions. By varying these in a suitable manner, Equations (V) may be employed to deduce with reasonable accuracy the changes in soil moisture to be expected in the tanks under a fairly wide range of environmental conditions. Extrapolation beyond the observed range of intensity of precipitation, namely from zero to about 1.25 in. per day, would not however be justifiable.

There must of course be some question as to the closeness with which the experimental tanks reproduce the moisture relations characteristic of an open field. Barnes (1) pointed out that initially at least the soil placed in them had been moved, stirred, and thoroughly aerated to a depth of 5 ft., and that in this process all columnar structure had been destroyed. Furthermore, the tank walls restrict the lateral movement of moisture and prohibit percolation beyond the 5-ft. depth or the upward movement of water or its vapour from below. Finally, the rims of the tanks minimize losses from surface run-off. He did not mention soil temperature, which may conceivably have been somewhat higher in the tanks, during summer at least, than at corresponding

levels in the field. It is admittedly difficult to estimate the combined effect of all these factors upon the behaviour of the tank system. Barnes himself apparently thought that on the whole conservation would be somewhat increased. The recent observations of Veihmeyer (10) on the behaviour of soil moisture in general, of Lewis (6) on the restricted role of capillary movement, and of Taggart (8) on the subject of run-off, suggest however that the resultant departure from field conditions is probably not as great as might at one time have been supposed.

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VEGETATIVE PROPAGATION OF CONIFERS

VI. HORMONE SOLUTION AND DUST TREATMENTS OF SPRUCE CUTTINGS PROPAGATED IN GREENHOUSE AND OUTSIDE FRAMES[1]

By N. H. Grace[2] and J. L. Farrar[3]

Abstract

Four monthly collections of dormant Norway spruce cuttings, January to April, were treated with indolylbutyric acid solutions and propagated in sand in a greenhouse. February and April collections gave better rooting than those of the other two months, while there was appreciably greater mortality of the March and April collections. The results with short cuttings were uniformly superior to those obtained with long cuttings. Other cuttings of the April collection were propagated outside. The short cuttings responded more favourably to outside conditions, while the long cuttings gave equally poor results inside and outside. Indolylbutyric acid treatment had no beneficial effect and was followed by reduced rooting and increased mortality at concentrations from 20 to 60 p.p.m.

Results of a late March collection of Norway, white, and black spruce cuttings treated with talc dusts containing indolylacetic acid, cane sugar, and organic mercury, indicated that Norway spruce rooted more readily than the two other species. Treatment failed to have a beneficial effect, although injury from indolylacetic acid was somewhat reduced by its combination with organic mercury.

The results of these and the other experiments reported indicate that short Norway spruce cuttings over the period from January to April root to the extent of about 50%. A May collection, an early June collection with new growth on the cuttings, and a late June collection in which the cuttings were made from new growth only, gave inappreciable rooting. Similar new-growth cuttings did, however, give some rooting when propagated in sand watered with nutrient salts.

A number of papers have reported the results of experiments on the vegetative propagation of Norway spruce (*Picea abies* Karst) cuttings (3–11, 13). Among the factors having a bearing on rooting are the stage of development at which the cuttings were taken, age of the wood in the cutting, age of the parent tree and position of the cutting on it, plant hormone chemicals and the method of application, the effects of nutrient and disinfectant treatments, and the conditions under which propagation was carried out. The present communication describes the results of a series of six experiments which provide further information on the effects of some of these factors on the rooting of spruce cuttings.

Experimental

All the spruce cuttings used in the six experiments to be described were collected from the Petawawa Forest Experiment Station, Chalk River, Ontario. The Norway spruce cuttings were obtained from a plantation of trees 19 years of age. Four monthly collections of branches, January to April, were made by the staff of the Forest Station and shipped to Ottawa by express; these

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branches provided material for Experiments 1 to 4. In Experiment 4 use was made of the March, and in Experiments 2 and 3 of the April, collections of Experiment 1.

Material for Experiments 5 and 6 was transported to Ottawa by automobile. The white spruce cuttings were taken from a somewhat younger plantation, while the black spruce were from trees of approximately the same age in a natural upland stand.

The experiments were carried on in a greenhouse during the winter months and in outdoor frames in the late spring. The propagation medium was builder's sand, and all frames were shaded with factory cotton screens. Raised benches, equipped with electric heating cables in the media, were used in the greenhouse. Outdoors the sand rested directly on the soil and no bottom heat was provided. The greenhouse temperatures were maintained around 65° F. during the winter, but, beginning in April, temperatures ranged considerably higher and averaged about 72° F.

After a suitable period in the medium the cuttings were lifted and observations made in respect of: number of cuttings dead, number callused, number rooted, number of roots, and length of roots. From the last three quantities were calculated the average number of roots per rooted cutting, the average length of root per rooted cutting, and the mean root length per group. Data on the number of cuttings dead, callused, and rooted were subjected to the inverse sine transformation to render the values suitable for analysis of variance (2).

All experiments were arranged according to the principles of experimental design with all the treatments replicated and the groups arranged in random order in the medium. All results were subjected to the analysis of variance procedure except where the data were too meagre to warrant such treatment.

Experiment 1

This experiment was designed to complement one carried out at Yale University, New Haven, Connecticut, in which four collections, October to January, of Norway spruce cuttings were treated with a series of indolylbutyric acid solutions (3, 4, 5). The present experiment involved four monthly collections, January to April, and use was made of an identical experimental arrangement, except that it was limited to cuttings with a heel. There were 10 treatments, namely, an untreated control, a water-treated control, and applications of indolylbutyric acid solutions containing 0.625, 1.25, 2.5, 5, 10, 20, 40, and 60 p.p.m. (parts of chemical to a million parts of distilled water). Cuttings were made from two length classes of twigs, the short comprising lengths from 5 to 10 cm. and the long from 10 to 20 cm. There were five cuttings per group and four replicates, making a total of 400 cuttings to the collection, with 1600 cuttings required for the experiment.

The branches were received in the third week of the month, whereupon cuttings were prepared and treated as soon as possible in the solutions for a

period of 24 hr. Forty cuttings were treated in 200 cc. of solution; after treatment they were rinsed and planted immediately.

After approximately 14 weeks the cuttings were removed and record was made of the number rooted and dead.

Experiment 2

The material and treatments were identical with those of Experiment 1, except that the treatments were increased by one concentration, namely, 100 p.p.m. indolylbutyric acid. This series of 440 cuttings was propagated outdoors.

These cuttings were given a preliminary examination approximately 14 weeks after planting, but at this time rooting was poor. Numerous living cuttings failed to root, although a number were callused. In consequence, complete data were not taken until 24 weeks after planting.

Experiment 3

Norway spruce cuttings of an April collection were treated with talc dusts containing indolylacetic acid. The experiment comprised an untreated control, treatment with talc only, and with talc containing 100 and 1000 p.p.m. of indolylacetic acid. The cuttings were short (see Experiment 1) and had a heel of 1937 wood. They were planted outdoors in the same propagation frame as the cuttings of Experiment 2. There were 15 cuttings to a group, five replicates, and the entire experiment involved the use of 300 cuttings. The cuttings were removed for counts and measurements in mid-August, approximately 15 weeks after planting.

Experiment 4

In this experiment the cuttings were treated with a factorial series of eight dusts containing indolylacetic acid at 0 and 1000 p.p.m., cane sugar at 0 and 10%, and ethyl mercuric phosphate at 0 and 50 p.p.m. The carrier dust was talc. These treatments were part of the factorial series applied to a November collection of Norway spruce and already reported (9). In the present experiment the concentration of indolylacetic acid that had been shown to have a beneficial effect previously was used. The experiment was designed to demonstrate any change in the response to chemical treatments or media. A March collection of Norway spruce was used and cuttings ranged from 5 to 9 cm. in length. These cuttings were made with a heel of old wood, whereas plain cuttings were used in the earlier experiment (9). There were ten cuttings to a group and six replicates, three in sand and three in a mixture of sand and peat (equal proportions by volume). The propagation frames were in a greenhouse.

The same eight treatments, of the factorial series, were applied to March collections of both white and black spruce (*Picea mariana* (Mill) B.S.P. and *Picea glauca* (Moench.) Voss). Cuttings of white spruce were of approximately the same lengths as the Norway, while the average for the black spruce was about 6 cm. Cuttings had a heel of old wood and there were the same number

in each group and the same number of replicates, again three in each medium. The three blocks of each of the three spruces were randomized in each medium. This statistical arrangement gave the greatest precision for the comparison of treatments within species and a lower order of precision between species. The entire experiment involved the use of 1440 cuttings, 480 of each species. The cuttings were removed and examined approximately 14 weeks after planting (April 1).

Experiment 5

Dormant Norway spruce cuttings were collected from the lower region of the tree on May 15, 1939, and treated and planted the following day. The lengths and kind of cutting and the treatments were identical with those of Experiment 4, but there were four replicates of the eight treatments, 10 cuttings to a group, and all were planted in sand outdoors in a propagation bed similar to that used in Experiments 2 and 3. The entire experiment required 320 cuttings, which were removed for examination approximately 17 weeks after planting, when the usual records were taken.

Experiment 6

This experiment involved the use of an outside propagation frame containing 24 small adjacent compartments, each with an area of 1.5 sq. ft., filled with brown sand. These 24 compartments comprised 12 pairs, one of which, chosen at random, received a weekly application of one litre of a nutrient solution (7), the others receiving water only. This experimental arrangement provided 12 replicates of the treatment with and without the nutrient solution. Within each compartment three groups of five cuttings for each experiment were placed in random order; these comprised an untreated control group, a talc control, and a treatment with 1000 p.p.m. indolylacetic acid in talc. Two separate collections of cuttings were treated in this way. Both collections were taken from the lower region of the tree, and plain cuttings, without a heel, were used. The first was made June 9, 1939, and the cuttings ranged in length from 6 to 9 cm., each bearing a new growth shoot of approximately 1 cm. in length. The second collection was made June 21 and only material of the current year's growth was utilized; these cuttings ranged from 5 to 8 cm. in length. The cuttings were examined and records taken approximately 12 weeks after planting.

Results

The statistical treatment of the data is given in detail for Experiments 1 and 2, but in the remaining four experiments it was not included.

Experiment 1. Four monthly collections of Norway spruce cuttings treated with solutions of indolylbutyric acid, and propagated in a greenhouse

Results of the analyses of variance for the number dead and rooted after treatment are given in Table I. The effects of period of collection and indolylbutyric acid treatments are highly significant. There were significant differences in the behaviour of long and short cuttings as shown by both sets of

TABLE I

EXPERIMENT 1

ANALYSIS OF VARIANCE OF RESPONSE OF FOUR MONTHLY COLLECTIONS, JANUARY TO APRIL, OF NORWAY SPRUCE CUTTINGS TREATED WITH SOLUTIONS OF INDOLYLBUTYRIC ACID

Source of variance	Degrees of freedom	Mean square	
		Number of cuttings	
		Dead	Rooted
Rows	3	4083.21**	3133.35**
Columns	3	1525.07*	993.64
Collections	3	7421.26***	4956.39**
Error *(a)*	6	274.16	284.77
Long versus short cuttings	1	13411.02*	5764.71*
Interaction			
Long versus short × collection	3	879.52	476.51
Error *(b)*	12	1874.79	849.15
Treatments	9	2776.12***	1087.95**
Interaction			
Treatments × collections	27	481.36*	226.91
Treatments × long versus short cuttings	9	448.06	278.17
Treatments × long versus short × collection	27	314.51	137.15
Error *(c)*	216	245.55	166.72

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

observations. There was a significant interaction between hormone treatments and time of collection, but only for the number of cuttings dead. The nature of these significant effects is illustrated in the succeeding tables.

In Table II are given data for the number of cuttings dead following each treatment at each monthly collection. Data are given in percentage as well as in the transformed condition, and the values are averages for both short and long cuttings. The effect of month of collection, averaged over all hormone treatments, was 20% greater death for March and April than for January and February collections. The effect of indolylbutyric acid treatments, averaged over all four collections, was one of injury; the number of dead cuttings for all treatments, including water only, was greater than that for the untreated control. The three highest indolylbutyric acid concentrations caused greater death than did water only, while the results with the other treatments did not differ significantly from those with water only. The 60 p.p.m. treatment was followed by the death of 84% of the cuttings, a value greater than that of all other treatments. When the four collections were not treated, they did not differ significantly in behaviour. The water controls were progressively poorer as they were taken later in the season. The March and April collections showed a rapidly increasing death count with increasing indolylbutyric acid dosage; in January and February,

TABLE II

EXPERIMENT 1

NUMBER OF CUTTINGS DEAD OF FOUR COLLECTIONS OF NORWAY SPRUCE ON TREATMENT WITH SOLUTIONS OF INDOLYLBUTYRIC ACID

Each value in the body of the table is a mean of 8 groups of 5 cuttings

Month of collection	Kind of data	Untreated	Water only	Concentration of indolylbutyric acid, p.p.m.								Means by month of collection
				0.625	1.25	2.5	5	10	20	40	60	
January	Transformed	35.6	46.3	57.4	59.4	41.7	34.1	43.4	56.0	52.9	65.5	49.22
	%	37.5	52.5	67.5	67.5	47.5	35.0	47.5	65.0	60.0	77.5	55.8
February	Transformed	40.1	48.2	35.5	40.1	42.1	51.5	37.1	53.1	61.2	65.8	47.45
	%	45.0	55.0	37.5	45.0	45.0	57.5	40.0	57.5	67.5	75.0	52.5
March	Transformed	38.7	54.7	49.9	60.9	70.5	68.5	75.4	77.0	76.9	85.1	65.75
	%	42.5	60.0	55.0	70.0	80.0	82.5	85.0	87.5	87.5	95.0	74.5
April	Transformed	43.9	70.5	65.3	62.7	65.5	54.4	57.8	70.5	72.1	78.5	64.12
	%	45.0	80.0	77.5	70.0	77.5	62.5	65.0	80.0	82.5	90.0	73.0
Mean over the four collections	Transformed	39.6	54.9	52.0	55.8	54.9	52.1	53.4	64.1	65.8	73.7	
	%	42.5	61.9	59.4	63.1	62.5	59.4	59.4	72.5	74.4	84.4	

Necessary differences 5% level; treatment means 7.72; interaction treatments × collection 15.45; means by month of collection 6.41.

increasing dosage did not produce injury at the same rate, and the results were more irregular.

The mortality of the short cuttings was less than that of the long; 56% of the former and 72% of the latter were dead at the end of the experiment.

In Table III are given data for the effects of treatments on the number of cuttings rooted. These are averages for all four collections and for both long

TABLE III

EXPERIMENT 1

NUMBER OF CUTTINGS ROOTED OF FOUR COLLECTIONS OF NORWAY SPRUCE ON TREATMENT WITH SOLUTIONS OF INDOLYLBUTYRIC ACID

Each value in the table is a mean of 32 groups of 5 cuttings

Kind of data	Untreated cuttings	Water only	Concentration of indolylacetic acid, p.p.m.							
			0.625	1.25	2.5	5	10	20	40	60
Transformed	18.0	9.4	20.8	13.5	18.3	16.8	18.0	8.5	9.5	2.5
%	16.9	8.8	20.0	12.5	17.5	15.6	17.5	8.8	8.1	1:9

Necessary difference, 5% level: 6.4.

and short cuttings. Treatment in water alone gave rooting below that of the untreated controls. The results with indolylbutyric acid treatments from 0.625 p.p.m. up to 10 p.p.m. did not differ from those of the untreated control. The three highest concentrations resulted in rooting below that of the untreated cuttings, while the rooting with the 60 p.p.m. treatment was also significantly below that with water only.

Data for the effects of month of collection on rooting are given in Table IV and are averages for all treatments and both lengths of cutting. A greater percentage of rooted cuttings was obtained from the February and April collections than from those made in January or March.

TABLE IV

EXPERIMENT 1

EFFECT OF MONTH OF COLLECTION ON ROOTING OF NORWAY SPRUCE CUTTINGS

Each value in the table is a mean of 80 groups of 5 cuttings

Month of collection	Number of cuttings rooted	
	Transformed data	Per cent
January	6.9	6.0
February	23.1	22.8
March	7.2	6.3
April	16.8	16.0

Necessary difference, 5% level of significance: 6.5.

Averaged over all treatments and collections, the short cuttings rooted to the extent of 17.3%, the long 8.3%.

Experiment 2. April collection of Norway spruce cuttings treated with solutions of indolylbutyric acid and propagated outside

Data from the analyses of variance of the number of cuttings dead, rooted, and alive but not rooted are given in Table V. The analyses of variance for the number and length of roots per rooted cutting and the mean root length are not given as analysis was confined to the short cuttings only, owing to indifferent rooting of the long cuttings and numerous missing values. These root counts and lengths failed to show any significant effects. Indolylbutyric acid treatment affected the number of cuttings dead, rooted, and alive but not rooted, in a significant manner. There were differences between the behaviour of long and short cuttings in relation to both the number dead and the number rooted; further, the number of rooted cuttings demonstrated the difference in the response of the two length classes of cuttings to treatment.

In Table VI are given data for the number of cuttings dead, rooted, and the number alive but not rooted. The values are averages based on both

TABLE V

EXPERIMENT 2

ANALYSIS OF VARIANCE OF RESPONSE OF AN APRIL COLLECTION OF NORWAY SPRUCE CUTTINGS PROPAGATED OUTDOORS AFTER TREATMENT WITH INDOLYLBUTYRIC ACID SOLUTIONS

Source of variance	Degrees of freedom	Mean square		
		Number of cuttings		
		Dead	Rooted	Alive but not rooted
Replicates	3	164.6	285.8	16.0
Short vs. long cuttings	1	16265.6**	8067.9**	3225.9
Error *(a)*	3	236.9	99.3	663.6
Treatments	10	2084.2**	1165.3**	485.2*
Interaction Short vs. long × treatments	10	390.3	525.3*	217.8
Error *(b)*	60	282.8	253.4	237.7

** Exceeds mean square error, 5% level of significance.*
*** Exceeds mean square error, 1% level of significance.*

TABLE VI

EXPERIMENT 2

RESPONSES OF AN APRIL COLLECTION OF NORWAY SPRUCE CUTTINGS TREATED WITH SOLUTIONS OF INDOLYLBUTYRIC ACID AND PROPAGATED OUTDOORS

Each value in the table is a mean of 8 groups of 5 cuttings

Number of cuttings	Kind of data	Untreated	Water only	Concentration of indolylbutyric acid, p.p.m.								
				0.625	1.25	2.50	5.0	10.0	20.0	40.0	60.0	100.0
Dead	Transformed	42.0	38.5	36.9	38.7	44.7	54.4	48.2	55.8	67.2	72.3	86.7
	%	45.0	42.5	42.5	42.5	52.5	62.5	55.0	65.0	77.5	80.0	97.5
Rooted	Transformed	27.7	34.1	30.7	37.1	30.7	26.1	29.3	18.0	6.7	6.7	3.3
	%	27.5	35.0	32.5	40.0	32.5	25.0	30.0	17.5	5.0	5.0	2.5
Alive, not rooted	Transformed	27.8	24.7	28.0	21.5	16.5	14.9	18.2	19.8	16.2	14.6	0
	%	27.5	22.5	25.0	17.5	15.0	12.5	15.0	17.5	17.5	15.0	0

Necessary differences, 5% level, transformed data only; number of cuttings dead 16.8, rooted 15.9, alive but not rooted 15.4.

long and short cuttings. The three highest concentrations of indolylbutyric acid, namely, 40, 60, and 100 p.p.m., resulted in more dead cuttings than either the untreated or water control groups. None of the indolylbutyric acid treatments from 0.625 p.p.m. to 10 p.p.m. inclusive, differed from either of the control groups in respect to the number of cuttings dead. There were

no significant differences in rooting between the control groups and any of the groups treated with 0.625 to 10 p.p.m.; however, treatments from 40 to 100 p.p.m. reduced rooting below that of the controls and were below that for all other treatments, except at 20 p.p.m. The number of cuttings alive but not rooted failed to differ significantly except after 100 p.p.m. treatment, which resulted in no such living cuttings.

In Table VII are given data for the rooting of short and long cuttings after the various treatments. The short cuttings gave significantly better rooting for both control groups and for treatment from 0.625 p.p.m. up to 10 p.p.m. indolylbutyric acid, excluding the 5 p.p.m. treatment. For treatments from 20 up to 100 p.p.m. of indolylbutyric acid, injury was marked and there was no significant difference in the response of the two lengths of cutting.

TABLE VII

EXPERIMENT 2

DIFFERENTIAL ROOTING RESPONSE OF LONG AND SHORT NORWAY SPRUCE CUTTINGS TO TREATMENT WITH SOLUTIONS OF INDOLYLBUTYRIC ACID

Each value in the table is a mean of 4 groups of 5 cuttings

Kind of cuttings	Kind of data	Untreated	Water only	Concentration of indolylbutyric acid, p.p.m.								
				0.625	1.25	2.50	5.0	10.0	20.0	40.0	60.0	100.0
Short	Transformed	42.1	48.2	51.7	51.1	48.2	23.1	45.3	19.6	6.7	13.3	6.7
	%	45	55	55	60	55	20	50	20	5	10	5
Long	Transformed	13.3	19.9	9.8	23.1	13.3	29.2	13.3	16.5	6.7	0	0
	%	10	15	10	20	10	30	10	15	5	0	0

Necessary difference, 5% level (transformed data) 22.5.

The short cuttings were rooted to a greater extent and there were fewer dead. Considering averages over all the treatments, 11 and 78% of the long cuttings were rooted and dead, respectively; the comparable results with short cuttings were 34% rooted and 42% dead. It is of interest to point out that of the April collection propagated in the greenhouse (Experiment 1), 10% of the long cuttings rooted and 81% were dead, while 22% of the short rooted, with 64% dead. These results suggest that there is little difference in either the rooting or death of the long cuttings, whether propagation occurs inside in a greenhouse or outside. However, outside conditions appear to be somewhat favourable for the short cuttings.

Experiment 3. April collection of Norway spruce cuttings treated with indolylacetic acid in talc dusts and propagated outside

The only significant treatment effect obtained on analysis of the data for the number of cuttings rooted was between the untreated control group, which rooted 56%, and the three talc dusted groups, which rooted approximately 22%. Indolylacetic acid treatment failed to affect the number of

cuttings rooted. Essentially the same treatment differences were shown by counts of the number of cuttings callused or rooted. While approximately 87% of the untreated cuttings were either rooted or callused, the average for the three talc dusted groups was 59%. Indolylacetic acid treatment did not affect the number of cuttings rooted, callused, or dead. However, while only 7% of the untreated cuttings were dead, approximately 20% of those dusted with talc, the average of the three talc treatments, had died during the course of the experiment.

There were no significant treatment effects shown by the data for the lengths of root per rooted cutting. However, untreated cuttings had an average of 1.8 roots per rooted cutting and the average for the three talc treatments was 1.5 roots, indolylacetic acid treatment having no effect. Significant differences on the mean root length were confined to the three talc treatments. The 100 p.p.m. indolylacetic acid treatment gave a mean root length of 10.6 mm., while the talc only and the 1000 p.p.m. gave an average of 20.2 mm.

Experiment 4. Spruce cuttings treated with talc dusts containing indolylacetic acid, cane sugar, and ethyl mercuric phosphate, and propagated in a greenhouse

All white and black spruce cuttings in the mixture of peat and sand died. About 8% of the black spruce planted in sand callused and none rooted, and only 7% of the white spruce cuttings in sand were callused and one cutting of the 240 was rooted. In consequence, it was impossible to make any statistical analysis of such meagre data, and the analyses were confined to data for the Norway spruce cuttings.

There were significant differences between the two media in respect of all counts. In sand, there were 23% of rooted cuttings, 39% of rooted and callused, and 54% dead; in the mixture of sand and peat the counts were, respectively, 6, 20, and 76%. Peat sand gave uniformly poorer results than sand only.

Data for the number of cuttings rooted failed to disclose any significant treatment effects. However, there were some significant effects shown by chemicals on the number of cuttings rooted and callused. Averaged over the results for both media, the three treatments with talc containing indolylacetic acid alone and in combination with cane sugar and organic mercury were below the treatment with talc only. The average number of cuttings rooted and callused following the three preceding treatments was 20%, while talc alone gave 45% of such cuttings. The results with the other four treatments did not differ from those with talc only. The average effect of indolylacetic acid was depressing. In its absence 35% of the cuttings were rooted and callused, and in its presence 24%, these values being averages for the results in both media and for treatments with and without organic mercury and cane sugar. In Table VIII are given data for the effects of the combination of indolylacetic acid and the organic mercurial, these being averages

for the results in both media and for treatments with and without cane sugar. In the absence of organic mercury, indolylacetic acid was injurious and reduced the number of cuttings rooted and callused, but this injurious effect of indolylacetic acid disappeared in the presence of organic mercury.

TABLE VIII

EXPERIMENT 4

INTERACTION EFFECTS OF INDOLYLACETIC ACID AND ORGANIC MERCURY ON THE NUMBER OF CUTTINGS CALLUSED AND ROOTED AFTER TREATMENT WITH TALC DUSTS CONTAINING INDOLYLACETIC ACID, CANE SUGAR, AND ETHYL MERCURIC PHOSPHATE

Each value in the table is a mean of 12 groups of 10 cuttings

Concentration of indolylacetic acid, p.p.m.	Kind of data	Concentration of organic mercury, p.p.m.	
		0	50
0	Transformed	38.7	31.4
	%	41.7	28.3
1000	Transformed	22.3	31.3
	%	19.2	28.3

Necessary difference 5% level 8.75 (transformed data).

Averaged over the entire experiment, treatments failed to affect in a significant manner the number of cuttings dead. However, the interaction between indolylacetic acid and organic mercury treatment was significant. Data for the interaction effects are given in Table IX and are averages for the number of cuttings dead in both media and treatments with and without cane sugar. The number of dead cuttings was increased by indolylacetic

TABLE IX

EXPERIMENT 4

INTERACTION EFFECTS OF INDOLYLACETIC ACID AND ORGANIC MERCURY ON THE NUMBER OF CUTTINGS DEAD AFTER TREATMENT WITH TALC DUSTS CONTAINING INDOLYLACETIC ACID, CANE SUGAR, AND ETHYL MERCURIC PHOSPHATE

Each value in the table is a mean of 12 groups of 10 cuttings

Concentration of indolylacetic acid, p.p.m.	Kind of data	Concentration of organic mercury, p.p.m.	
		0	50
0	Transformed	48.0	56.8
	%	53.3	69.2
1000	Transformed	61.2	53.6
	%	72.5	64.2

Necessary difference, 5% level 9.29 (transformed data).

acid treatment in the absence, but not in the presence, of organic mercury, a condition analogous to that shown by similar data for the number of rooted and callused cuttings.

Data for the numbers and lengths of root were based on results in sand only, owing to poor rooting in peat sand and numerous missing values. The average number of roots per rooted cutting was 1.4 and the average length of roots per rooted cutting was 52.8 mm., but none of the treatments gave significantly different results.

Experiment 5. Dormant Norway spruce cuttings collected in May and propagated outdoors

There were few rooted cuttings, only 10 out of the total of 320 cuttings planted, but 83% were still alive. The number of cuttings dead failed to disclose any significant treatment effects. The striking feature of these results is the absence of appreciable rooting, especially since the cuttings were taken only three weeks after the collections of Experiments 2 and 3, collections that gave a fairly substantial degree of rooting.

Experiment 6. Effects of nutrient salts and indolylacetic acid treatments on June collections of Norway spruce cuttings propagated outdoors

The first collection, 1938 wood with 1939 growth, showed no signs of rooting, and virtually all the new growth shoots were dead. No data were taken on this collection.

Cuttings from the second collection, in which the cuttings were entirely of 1939 growth, likewise failed to root in appreciable quantities. While about 11% of both the untreated and those treated with 1000 p.p.m. indolylacetic acid rooted, 20% of those with talc only were rooted. These results are suggestive, but are not statistically significant.

Data on the number of cuttings dead indicated very highly statistically significant differences due to both nutrient and dust treatments. While 57% of the cuttings that received nutrient were dead, the value was 84% in its absence. The effect of treatments on the number of cuttings dead, averaged over the results with and without nutrient treatment, showed that, while about 78% of both the untreated and those which received 1000 p.p.m. indolylacetic acid were dead, only 55% of the talc-treated cuttings died.

Discussion

It has been shown that the rooting of spruce cuttings is affected by a number of factors (3-11, 13). There are other difficulties in interpreting and correlating the results of any series of experiments on vegetative propagation. One of the more important complicating factors is variation of the conditions under which propagation is carried out. Conditions in the greenhouse vary from month to month, and outside propagation one summer may encounter widely different conditions from those prevailing the next. Such variations must be considered in relation to the rooting response of different monthly

collections of cuttings. Comparison and correlation of the results of experiments by one, or several, investigators are complicated by these sources of variability, which, in conjunction with the natural variability of the material, render it necessary to exercise care in drawing general conclusions. However, in spite of variability of the types mentioned, certain general trends have been indicated by the results of the six experiments herein described.

Dormant Norway spruce cuttings taken in the period October to April can be rooted in appreciable numbers when propagated in a greenhouse; this finding is in agreement with earlier reports (3–11, 13). The optimum period for rooting appears to be from November to January. Unpublished work by both authors has indicated that Norway spruce cuttings do not root readily when collected during the summer months.

Application of growth stimulating chemicals by the solution method of treating cuttings has failed to demonstrate anything but injurious effects with Norway spruce. While this conclusion is in agreement with the previous findings of the authors and of Deuber and Farrar (3, 5, 10), it is not in agreement with reports from Thimann and Delisle, and Hitchcock and Zimmerman (11, 13). Application of indolylacetic acid by the dust method to April cuttings, likewise was injurious to rooting or had no effect, though beneficial effects from dust treatments of November cuttings have been reported (9). There appears to be variation in the effects of phytohormone treatment with the time of year at which the cuttings are taken.

The propagating medium is one of the more important factors affecting the rooting of cuttings. In an experiment previously reported, a beneficial effect was suggested from the addition of peat moss to the sand ordinarily used (9). In the present Experiment 4, carried out four months later, a significantly injurious effect resulted from the mixture. Chadwick, working with *Taxus* cuttings, also found a change in the effect of media as the season progressed (1).

The results of Experiment 4, and numerous others carried out at various times of the year, indicated that black and white spruce cuttings are much more difficult to root than are those of Norway spruce. However, conditions optimum for one species are not necessarily optimum for another, and there may be conditions under which both black and white spruce cuttings will root with ease.

An interesting feature of the dust method of treating cuttings is the beneficial effect of the carrier dust (7, 11, 12). In Experiment 6, reduced mortality from treatment with talc only was not influenced by the application of nutrient salts to the propagating medium. This fact and the beneficial effect of charcoal reported by Rappaport (12) suggest that the action of the carrier dust is due to its physical properties rather than its chemical constituents. In Experiment 3, talc had an injurious effect, and this is probably another instance of the differential response to treatments under different conditions.

The favourable effects of watering new growth cuttings with a solution of nutrient salts in Experiment 6 are in agreement with the findings from similar treatment of dormant cuttings (7).

Short cuttings have rooted in greater numbers and with lower mortality than the long. This superiority of the short type of cutting has been shown consistently in all experiments at Ottawa and at the Petawawa Forest Experiment Station, Chalk River. This is in marked contrast to the superior results obtained from long cuttings by Deuber and Farrar at New Haven, Connecticut (3–5).

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CANADIAN WILTSHIRE BACON

XII. EFFECT OF HEAT TREATMENT ON THE COLOUR AND COLOUR STABILITY OF BACON[1]

BY C. A. WINKLER[2] AND J. W. HOPKINS[3]

Abstract

The colour of quadruplicate pieces of factory-cured bacon heated at temperatures of 20, 40, 50, 60, 70, and 80° C. for 5, 10, 20, and 40 hr. was measured at the conclusion of heating, and also after subsequent exposure of the samples for 12, 20, and 96 hr. at 10° C. and 95% relative humidity. Interacting effects of time and temperature of heating on colour at the conclusion of treatment were demonstrable. At 40 and 50°, total intensity increased with the duration of heating; at 60 and 70°, there was no definite trend, and at 80° it diminished markedly as the period of heating was prolonged. The maximum average intensity resulted from heating at 70°.

The decrease in intensity after 12 hr. exposure was related to the duration, but not to the temperature, of previous heating. There was no significant change in intensity between 12 and 20 hr., but a further decrease was evident at the end of 96 hr. The decrease in green intensity was still related to the duration, rather than to the temperature, of heat treatment, but the effects of duration of heating on red and blue stability, noted at 12 and 20 hr., were now replaced by temperature effects.

Partial correlation coefficients indicate that increased nitrite content of the meat at the conclusion of heating tended to be associated with a lower intensity of colour. On the other hand, both nitrite content and loss in weight (chiefly moisture) on heating were correlated with increased colour stability on exposure.

In the preceding paper of this series (2), White, Cook, and Winkler describe the details of an experiment designed to investigate the effect of heating Wiltshire bacon, as in smoking, for various periods at different temperatures, on its nitrite content and colour. The experiment comprised four periods of heating, namely, 5, 10, 20, and 40 hr., and six temperatures, 20, 40, 50, 60, 70, and 80° C., or 24 treatments in all. Four out of 16 "backs" cut from individual factory-cured sides were allotted at random to each of the four periods of heating, and each back was subdivided into six portions which were allocated, also by a random process, to the six temperatures maintained for each period of time. The above-mentioned authors conclude that heating at 55° C. resulted in an approximately normal nitrite content. Temperatures

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below 55° resulted in increased amounts of nitrite being found on analysis, whereas higher temperatures diminished the concentration of this constituent. These effects of temperature were accentuated by prolonging the time of heating.

It is now desired to describe the results of colour measurements of the 96 individual samples after treatment, secured by means of the photoelectric comparator employed in other phases of this investigation, already described elsewhere (3). The effect of heat treatment on colour and colour stability will first be dealt with, after which the correlation between these colour measurements and the foregoing determinations of nitrite content will be discussed.

Effect of Heat Treatment on Colour

The photoelectric comparator was employed as before to determine the component intensities of red, green, and blue light, relative to the white standard, reflected or scattered from the surface of the cut samples (4), and the sum of these three relative values was again taken as the index of colour intensity or total brightness.

Intensity or Total Brightness

Table I shows the results of an analysis of the variance (1) of the colour intensity as defined above, at the conclusion of heating of the 80 individual samples treated at temperatures of 40 to 80° C., and Table II the treatment averages, as well as the means by periods, for all temperatures, and by temperatures, for all periods. As was explained by White, Cook, and Winkler (2) the effect of temperature, which is deduced from comparisons between subsamples of the same sides, is more accurately determined than that of period of heating, which is affected by the variability between sides. This is reflected in the magnitude of the mean square errors *(a)* and *(b)* in Table I.

The analysis of variance demonstrates a highly significant effect of temperature on subsequent intensity of colour, and reference to Table II will indicate that the mean intensity, averaged for all periods, increased with

TABLE I

ANALYSIS OF VARIANCE OF COLOUR INTENSITY OR TOTAL BRIGHTNESS OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square
Period of heating (all temps.)	3	261.0
Error *(a)* (between sides)	12	303.8
Temperature (all periods)	4	3,273.0***
Interaction, temp. × period	12	618.6***
Error *(b)* (within sides)	45	39.0

****Exceeds mean square error, 0.1% level of significance.*

TABLE II

AVERAGE COLOUR INTENSITY OR TOTAL BRIGHTNESS OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	121	120	126	154	163	168	146
10	115	115	134	155	163	158	145
20	113	128	139	152	157	143	144
40	—	143	158	161	169	128	152
Mean	116	126	139	156	163	149	147

temperature from 40 to 70°, falling off again however at 80°. The means for the four periods of heating, when taken over all five temperatures, do not differ significantly, but there is a significant interaction of temperature and duration of heating. Thus at 40 and 50°, the samples exhibited an increasing intensity of colour as the duration of treatment was prolonged. At 60 and 70° there was no appreciable trend, and at 80° the trend was the reverse of that noted at 40 and 50°, the brightness diminishing markedly with length of heating at this temperature.

For the reasons previously stated (2), the observations made on the samples heated at 20° C. were not included in the foregoing analysis of variance. A separate examination of these data did not indicate any significant difference in the brightness of the samples held for 5, 10, and 20 hr. at this temperature.

Chroma or Colour Quality

Tables III, IVA, IVB, and IVC give the analyses of variance and average values of the component red, green, and blue intensities of the samples subjected to the various treatments, in the same form as that adopted in Tables I and II above.

TABLE III

ANALYSIS OF VARIANCE OF COMPONENT INTENSITIES OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square		
		Red	Green	Blue
Period of heating (all temps.)	3	39.7	50.5	30.3
Error *(a)* (between sides)	12	29.9	33.3	28.9
Temperature (all periods)	4	558.3***	336.2***	404.1***
Interaction, temperature × period	12	94.5***	61.7***	15.5
Error *(b)* (within sides)	45	9.6	5.1	11.6

****Exceeds mean square error, 0.1% level of significance.*

TABLE IV. A

AVERAGE INTENSITY OF RED COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	49.0	46.0	49.0	61.0	64.9	65.6	57.3
10	46.4	46.0	53.4	62.7	65.7	61.4	57.9
20	43.7	50.8	53.9	61.0	62.4	57.8	57.2
40	—	57.2	62.7	63.8	66.5	50.9	60.2
Mean	46.3	50.0	54.8	62.1	64.9	59.0	58.2

The analyses of variance of red and green, and the treatment averages shown in Tables IVA and IVB, both reproduce features already noted in the results for total brightness, namely an increase in the average intensity for all periods of treatment with temperature up to 70°, followed by a reduction at 80°, and a significant interaction of temperature and treatment, intensity of colour increasing with duration of heating at 40 and 50°, and decreasing at 80°. There might seem to be some difference in the behaviour of the blue component, for its interaction mean square in Table III does not attain

TABLE IV. B

AVERAGE INTENSITY OF GREEN COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	37.9	37.3	39.3	46.1	50.0	52.6	45.1
10	35.4	35.2	41.2	47.5	50.4	48.7	44.6
20	35.4	39.4	42.0	46.7	48.9	44.7	44.4
40	—	44.2	49.4	51.1	54.2	40.0	47.8
Mean	36.2	39.0	43.0	47.9	50.9	46.5	45.4

significance. Reference to Table IVC will, however, indicate that there is in fact some reversal of the time effect at the high and low temperatures, but that the effects of treatment in general are less pronounced in this component, and that the differences between the intensities recorded after heating for 5 and 10 hr., and between those for 20 and 40 hr., in particular, are for the most part quite insignificant. These comparisons, which contribute eight of the total 12 degrees of freedom for interaction, dilute the variance computed from the data as a whole, and when the remaining four degrees of freedom, representing the average interaction, with temperature, of heating for 5 and 10 hr. on the one hand and for 20 and 40 hr. on the other were isolated, they

TABLE IV. C

AVERAGE INTENSITY OF BLUE COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	33.8	36.4	37.4	47.0	47.9	49.6	43.6
10	33.3	33.8	39.7	44.9	46.6	47.4	42.5
20	34.3	37.4	42.8	44.3	46.1	35.5	41.2
40	—	42.2	45.8	46.0	48.5	37.0	43.9
Mean	33.8	37.4	41.4	45.5	47.3	42.4	42.8

yielded a mean square of 34.9, significantly in excess of the mean square error *(b)*, 11.6. It is to be concluded, therefore, that the treatments imposed influenced all three colour components in a similar manner, but that the magnitude of the effects was greatest in the red and least in the blue region.

The samples maintained at 20° C., which were again considered separately, failed to exhibit any statistically significant differences attributable to the period of heating.

Effect of Heat Treatment on Colour Stability

In addition to the foregoing observations at the conclusion of heat treatment, further measurements of the intensity of the red, green, and blue components of colour were made after the treated samples of bacon had been exposed in a light-proof chamber for 12, 20, and 96 hr., at 10° C. and 95% relative humidity. These enabled the effects of heat treatment on colour stability, which will again be considered under the separate heads of total intensity and colour quality, to be followed in some detail.

Intensity or Total Brightness

Tables V and VI summarize the results of the observations of change in total intensity as defined above, the former giving the analyses of variance (40 to 80°), and the latter the treatment averages for the 12-, 20-, and 96-hr. periods of exposure.

It will be noted at once from Table V that whereas the change in intensity after both 12 and 20 hr. exposure varied significantly in relation to the duration, but not the temperature, of heating, after 96 hr. these effects of heating period had disappeared. Curiously enough, the samples heated for the shortest periods showed the greatest decreases in intensity of colour after 12 hr. exposure (Table VI). There was no significant change in intensity between 12 and 20 hr.; in fact, the mean decrease observed after 20 hr., 5.2 units, was slightly less than the corresponding figure of 6.3 after 12 hr., but the difference is within the limits of the experimental error. By 96 hr., however, the mean decrease (at 19.0 units) was appreciably greater and, as noted above,

TABLE V

ANALYSIS OF VARIANCE OF CHANGE IN COLOUR INTENSITY OR TOTAL BRIGHTNESS ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square		
		12 hr. exposure	20 hr. exposure	96 hr. exposure
Period of heating (all temps.)	3	245.6***	323.3*	182.0
Error *(a)* (between sides)	12	18.9	58.7	60.8
Temperature (all periods)	4	11.7	9.9	135.6*
Interaction, temperature × period	12	44.0	40.3	43.2
Error *(b)* (within sides)	45	47.3	45.2	45.1

* *Exceeds mean square error, 5% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE VI

AVERAGE CHANGE IN COLOUR INTENSITY OR TOTAL BRIGHTNESS ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−10.6	−11.2	−14.4	−10.0	− 9.4	− 5.2	−10.0
12	10	− 3.2	− 2.4	−12.4	− 9.8	− 7.0	− 7.6	− 7.9
12	20	− 5.9	− 8.2	− 3.6	− 5.3	− 3.6	− 6.1	− 5.4
12	40	—	− 1.2	− 0.8	+ 1.4	− 3.0	− 5.7	− 1.9
12	Mean	− 6.6	− 5.8	− 7.8	− 5.9	− 5.7	− 6.2	− 6.3
20	5	− 7.0	− 7.2	−11.1	− 9.2	− 8.6	− 5.8	− 8.4
20	10	− 0.8	− 2.4	− 9.8	−10.6	− 8.8	− 7.6	− 7.8
20	20	− 2.3	− 5.1	− 1.4	− 7.9	− 3.8	− 7.3	− 5.1
20	40	—	− 0.8	+ 1.3	+ 5.6	− 0.8	− 3.2	+ 0.4
20	Mean	− 3.4	− 3.9	− 5.3	− 5.5	− 5.5	− 6.0	− 5.2
96	5	−15.2	−15.4	−20.7	−22.4	−22.7	−18.1	−19.9
96	10	− 7.9	− 7.6	−19.5	−21.8	−21.4	−22.2	−18.5
96	20	− 9.8	−14.8	−12.2	−18.6	−16.4	−13.7	−15.1
96	40	—	−21.0	−23.2	−22.5	−27.3	−17.8	−22.4
96	Mean	−10.9	−14.7	−18.9	−21.4	−22.0	−17.9	−19.0

the earlier effects of duration of heating had disappeared, but differences related to temperature had developed. These were such that the magnitude of the decrease in intensity increased with temperature of heating from 40 to a maximum at 70°, falling off again at 80°. There is thus a parallelism between the effects of temperature on colour at the conclusion of heating,

and on colour stability, which is worthy of remark in view of the correlation between initial intensity and stability noted elsewhere (5). In contrast to the effects on initial colour, however, there was no demonstrable interacting influence of temperature and duration of heating on stability.

Chroma or Colour Quality

Table VII gives the results of analyses of variance of the change in the component red, green, and blue intensities after exposure of the samples for 12, 20, and 96 hr. The results for the red and blue components resemble those already noted in the foregoing analysis of the changes in total intensity in indicating significant differences in stability after 12 and 20 hr. related to the duration of heating, which were replaced by differences ascribable to temperature at the end of 96 hr. Tables VIII and X further indicate that these effects were similar in nature to the ones observed in total intensity. Both of the components mentioned showed a greater reduction at 12 and 20 hr. following the shorter periods of heating, and a maximum decrease in intensity after 96 hr. in the samples heated at 70°.

TABLE VII

ANALYSIS OF VARIANCE OF CHANGE IN COMPONENT INTENSITIES OF COLOUR ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square (red)			Mean square (green)			Mean square (blue)		
		12 hr. exposure	20 hr. exposure	96 hr. exposure	12 hr. exposure	20 hr. exposure	96 hr. exposure	12 hr. exposure	20 hr. exposure	96 hr. exposure
Period of heating (all temps.)	3	26.6*	39.6*	23.1	18.0**	17.3	28.0*	47.6***	64.9**	22.0
Error *(a)* (between sides)	12	5.6	9.9	9.0	2.2	5.6	6.8	3.4	9.4	7.4
Temperature (all periods)	4	4.1	8.7	33.5*	1.7	1.3	5.6	5.8	3.0	18.4*
Interaction, temperature × period	12	12.3	9.5	8.9	5.2	4.9	3.5	4.7	4.7	6.0
Error *(b)* (within sides)	45	7.0	4.9	8.3	6.7	6.6	6.1	3.7	6.5	6.0

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

The changes in the green component, on the other hand (Table IX), followed a somewhat different course, perceptible effects of duration of heating persisting even after exposure of the samples for 96 hr., whereas temperature effects were confined to narrow limits, which did not exceed the experimental error. After 96 hr. exposure, however, the decrease in green intensity of the various samples was no longer inversely proportional to the duration of heating. There was still a progressive diminution in loss of intensity from

TABLE VIII

AVERAGE CHANGE IN RED INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−5.2	−3.2	−5.8	− 4.0	− 4.2	− 0.4	−3.5
12	10	−1.8	−1.0	−4.4	− 5.4	− 3.4	− 1.8	−3.2
12	20	−0.0	−2.6	−0.2	− 1.5	− 0.4	− 2.4	−1.4
12	40	—	−0.0	−0.4	− 0.6	− 2.2	− 3.0	−1.3
12	Mean	−2.3	−1.7	−2.7	− 2.8	− 2.5	− 1.9	−2.3
20	5	−4.1	−1.3	−4.5	− 4.0	− 4.7	− 1.8	−3.3
20	10	−1.6	−2.1	−4.8	− 5.5	− 4.8	− 3.4	−4.1
20	20	+0.6	−2.2	+0.3	− 4.4	− 2.2	− 4.1	−2.5
20	40	—	−0.6	−0.3	+ 0.8	− 1.7	− 2.2	−0.8
20	Mean	−1.7	−1.6	−2.3	− 3.2	− 3.4	− 2.9	−2.7
96	5	−7.7	−5.8	−7.9	− 8.9	− 9.9	− 7.2	−7.9
96	10	−4.4	−4.2	−8.3	−10.5	−10.8	−10.9	−8.9
96	20	−3.4	−7.0	−5.0	− 9.3	− 7.4	− 6.7	−7.1
96	40	—	−8.9	−9.2	− 9.6	−11.9	− 8.2	−9.5
96	Mean	−5.2	−6.5	−7.6	− 9.6	−10.2	− 8.2	−8.4

TABLE IX

AVERAGE CHANGE IN GREEN INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−4.1	−3.9	−3.3	−1.6	−2.5	−2.1	−2.7
12	10	−0.6	−0.4	−3.6	−2.2	−1.9	−1.6	−1.9
12	20	−2.4	−3.2	+0.1	−1.7	−0.6	−1.8	−1.4
12	40	—	−0.5	−0.5	+0.7	−0.6	−1.2	−0.4
12	Mean	−2.4	−2.0	−1.8	−1.2	−1.4	−1.6	−1.6
20	5	−2.6	−2.8	−2.2	−0.5	−0.8	−1.0	−1.5
20	10	+0.6	+0.1	−2.2	−1.8	−1.9	−0.4	−1.2
20	20	−1.4	−1.5	+0.5	−0.8	+0.2	−1.2	−0.6
20	40	—	+0.3	+1.1	+2.2	+0.2	−0.7	+0.6
20	Mean	−1.1	−1.0	−0.7	−0.2	−0.6	−0.8	−0.6
96	5	−5.2	−5.0	−5.7	−4.8	−5.9	−5.0	−5.3
96	10	−2.2	−1.9	−5.0	−5.4	−4.9	−4.1	−4.3
96	20	−3.0	−4.3	−3.0	−4.3	−3.3	−3.0	−3.6
96	40	—	−5.8	−7.0	−6.7	−7.4	−4.5	−6.3
96	Mean	−3.5	−4.3	−5.2	−5.3	−5.4	−4.2	−4.9

TABLE X

AVERAGE CHANGE IN BLUE INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−1.3	−4.2	−5.2	−4.4	−2.7	−2.8	−3.9
12	10	−0.8	−1.0	−4.4	−2.3	−1.7	−4.3	−2.7
12	20	−3.5	−2.4	−3.5	−2.1	−2.6	−1.9	−2.5
12	40	—	−0.7	+0.2	+1.3	−0.3	−1.4	−0.2
12	Mean	−1.8	−2.1	−3.2	−1.9	−1.8	−2.6	−2.3
20	5	−0.2	−3.1	−4.5	−4.8	−3.0	−3.0	−3.7
20	10	+0.1	−0.4	−2.8	−3.4	−2.0	−3.8	−2.5
20	20	−1.5	−1.5	−2.2	−2.6	−1.8	−2.0	−2.0
20	40	—	−0.5	+0.5	+2.5	+0.7	−0.3	+0.6
20	Mean	−0.5	−1.4	−2.2	−2.1	−1.5	−2.3	−1.9
96	5	−2.2	−4.5	−7.1	−8.7	−6.9	−5.8	−6.6
96	10	−1.3	−1.6	−6.2	−5.9	−5.7	−7.1	−5.3
96	20	−3.4	−3.5	−4.2	−5.0	−5.6	−4.0	−4.4
96	40	—	−6.3	−7.2	−6.2	−8.0	−5.0	−6.6
96	Mean	−2.3	−4.0	−6.2	−6.5	−6.6	−5.5	−5.7

the samples heated for 5 hr. to those heated for 20 hr., but, of all samples, the ones heated for 40 hr. now showed the maximum decrease. It is possible that after still further exposure, the decrease in green intensity following the various heat treatments might eventually have conformed to that of the other two components, but a further experiment would be required to test this point.

Correlation of Colour and Colour Stability with Moisture Loss and Nitrite Content

White, Cook, and Winkler having shown (2) that the nitrite content of the various samples was significantly affected by the heat treatment to which they were subjected, it was of interest to determine the extent to which changes in colour were related to the effects of heating on nitrite and moisture contents. These authors point out that the observed loss of weight of the samples on heating was an imperfect criterion of drying, as at the higher temperatures there was also some loss of melted fat which was absorbed by the paper wrapping. In the absence of more reliable data, however, the total loss in weight was of necessity adopted as the only available index of dehydration.

Table XI shows the coefficients of simple correlation between the loss in weight and logarithmic nitrite content (2) on the one hand, and the intensity and subsequent stability of colour on the other. There was a significant positive correlation between loss in weight and the intensity of red and green at the conclusion of heating, and a negative association between nitrite content

TABLE XI

COEFFICIENTS OF SIMPLE CORRELATION (*r*) BETWEEN MOISTURE LOSS AND NITRITE CONTENT, AND COLOUR AND COLOUR STABILITY OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Quantities correlated	*r*, intensity at conclusion of heating	*r*, change after exposure		
		12 hr.	20 hr.	96 hr.
Loss in weight × total intensity	+.26*	+.26*	+.10	+.09
Loss in weight × red intensity	+.28**	+.06	−.04	−.19
Loss in weight × green intensity	+.29**	+.16	+.10	+.06
Loss in weight × blue intensity	+.19	+.19	+.12	−.06
Log. nitrite content × total intensity	−.34**	+.07	+.23*	+.25*
Log. nitrite content × red intensity	−.37**	+.19	+.27*	+.27*
Log. nitrite content × green intensity	−.34**	−.10	−.01	+.04
Log. nitrite content × blue intensity	−.29**	+.12	+.09	+.20

**Exceeds 5% point ($r = \pm 0.20$).*
***Exceeds 1% point ($r = \pm 0.27$).*

and all three colour components, the latter indicating that samples above-average in nitrite content tended to have a lower intensity of colour. When the changes in colour on exposure were considered, the only significant correlations in evidence were those between loss in weight during heating and the change in total intensity after 12 hr. exposure, and between nitrite content and the change in red and in total intensity after 20 and 96 hr. All of these coefficients are positive, indicating that above-average loss in weight and nitrite content were associated with greater stability of colour on exposure.

In this connection, however, it is again necessary to take account of the fact that there was a significant negative correlation between the loss in weight and nitrite content of individual samples, and significant positive correlations between the individual red, green, and blue intensities at the conclusion of heating, as well as between the changes in each of these components in any particular sample on exposure. For this reason, the partial correlation coefficients shown in Table XII were computed.

The partial coefficients for total intensity at the conclusion of heating suggest that the apparent correlation of this quantity with loss in weight (Table XI) was a consequence of the negative association, noted above, between loss in weight and nitrite content, the latter alone significantly affecting total brightness. There does seem to have been a significant relation between loss in weight and the individual intensities of green and blue, but as the correlation was positive in one case and negative in the other, the resultant effect on total intensity was inappreciable. Nitrite content, on the other hand, may be judged to have had a demonstrable effect on total intensity, but apparently no selective action on the individual components of colour.

The partial coefficients also bring out relations between loss in weight and nitrite content, and the change in intensity of colour after 12 hr. exposure, not apparent from Table XI. In this case, there were significant effects not

TABLE XII

COEFFICIENTS OF PARTIAL CORRELATION (*r*) BETWEEN MOISTURE LOSS AND NITRITE CONTENT, AND COLOUR AND COLOUR STABILITY OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Quantities correlated	Independent of	*r*, intensity at conclusion of heating	*r*, change after exposure		
			12 hr.	20 hr.	96 hr.
Loss in wt. × total intensity	Log. nitrite content	+.04	+.42**	+.36**	+.37**
Loss in wt. × red intensity	Log. nitrite content, green and blue intensity	−.06	+.30**	+.12	−.13
Loss in wt. × green intensity	Log. nitrite content, red and blue intensity	+.22*	−.28**	−.11	+.11
Loss in wt. × blue intensity	Log. nitrite content, red and green intensity	−.22*	+.39**	+.20	+.07
Log. nitrite content × total intensity	Loss in weight	−.24*	+.35**	+.42**	+.43**
Log. nitrite content × red intensity	Loss in weight, green and blue intensity	−.16	+.45**	+.31**	+.14
Log. nitrite content × green intensity	Loss in weight, red and blue intensity	+.11	−.45**	−.24*	−.13
Log. nitrite content × blue intensity	Loss in weight, red and green intensity	−.05	+.43**	+.19	+.17

**Exceeds 5% point ($r = \pm 0.21$).*
***Exceeds 1% point ($r = \pm 0.27$).*

only on the change in total intensity, but also on the changes in red, green, and blue, each independent of the other two. This is a feature of some interest, for which no explanation seems to be readily forthcoming. It is also to be noted that additional loss in weight and nitrite content were associated with increased stability of red and blue over the 12-hr. period, but with a decreased stability of green.

Separate effects of nitrite content on red and green stability were still detectable in the observations made after 20 hr. exposure, but by 96 hr. these independent effects were no longer distinguishable, although there was still a significant correlation between nitrite content and the change in total intensity. Loss in weight during heating was likewise correlated with the total decrease in intensity up to 96 hr., but in this instance independent effects on the individual components apparently did not persist beyond 12 hr. of exposure. Both loss in weight, which was uncorrelated, and nitrite content, which was negatively correlated with the total intensity of colour at the conclusion of heating, were associated with enhanced colour stability on subsequent exposure.

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CANADIAN WILTSHIRE BACON

XIII. TENDERNESS OF BACON AND EFFECT OF HEAT TREATMENT ON TENDERNESS[1]

By C. A. Winkler[2] and J. W. Hopkins[3]

Abstract

Measurements made on uncooked samples of two factory-cured sides from each of 22 Canadian packing plants indicated significant differences between plants in respect of tenderness. The maximum average cutting force required by the product of an individual plant was more than three times the minimum plant average. The individual results however were fairly uniformly distributed over the observed range of variation, neither the maximum nor the minimum representing an isolated extreme. Partial correlation coefficients indicated a significant influence of pH of both pump and cover pickle, in conjunction with number of "stitches" and days in cure respectively, on tenderness, but no appreciable relation of this property to salt content, moisture content, nitrate content or pH of the meat, or number of days from curing to receipt at the laboratory, was demonstrable.

A second series of observations on samples heated at 20, 40, 50, 60, 70, and 80° C. for 5, 10, 20, and 40 hr., as in smoking, demonstrated significant interacting effects of the temperature and duration of heat treatment on tenderness. Excepting one notably anomalous result, the general tendency was for toughness to increase with the prolongation of temperatures of 20 to 50°, but to decrease with the duration of temperatures of 60 to 80°. On the average, the maximum toughness was observed after heating at 50° C.

Introduction

In the course of a study of factors affecting the quality of Canadian Wiltshire bacon (1), determinations of the tenderness of two series of samples were made by means of the apparatus designed for this purpose by one of the authors (6). The two series comprised (i) samples of two factory-cured sides from each of 22 Canadian packing plants, and (ii) samples, of the same origin as in (i), subjected to various heat treatments, as in smoking.

As indicated in a previously published description (6) the apparatus actually provides a measure of toughness rather than of tenderness, namely the work required to cut through a piece of meat of specified width and thickness. The operation of the apparatus was the same as that previously described (6), and the data presented as indices of the cutting force are again the areas under the curve traced by the recording pen of the instrument in each case, corrected for thickness of sample. As these are directly comparable, they may be regarded as "relative work", without conversion into standard energy units. It was however found that over the range encountered in this series,

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the cutting force required varied more nearly as the square than as the actual thickness of individual samples. A Least Squares solution, based on 105 observations, actually indicated an average increase of 1.82 in the logarithm of the work done per unit increase in log thickness. This relation was accordingly used, in preference to the proportional factor previously adopted (6), to correct the observations for deviations of the individual test slices from the standard thickness of 5.0 mm.

Tenderness of Commercial Sides

Determinations were made on uncooked specimens from each of the 44 sides in series (i). For this purpose a sample of the necessary size was taken from the ham muscle of each side on arrival at the laboratory, the usual precautions being observed to secure representative material free from obvious striations of connective tissue (6).

After correction for thickness, an analysis of the variance (4) of the 44 resulting determinations indicated that the differences between the averages of the two sides from individual plants were significantly greater than would be expected from the observed variability of sides from the same plant. Table I summarizes the results of these observations, from which it is to be noted that the maximum average cutting force required by the product of an individual plant was as much as three times the minimum plant average. The individual results were however fairly symmetrically distributed over the observed range of variation, neither the maximum nor the minimum being an isolated extreme.

TABLE I

SUMMARY OF MEASUREMENTS OF TOUGHNESS, CORRECTED FOR THICKNESS, ON SAMPLES FROM 44 COMMERCIAL SIDES

Toughness	Relative cutting force required
Maximum	
Individual side	4.2
Plant (average of 2 sides)	3.6
Minimum	
Individual side	0.9
Plant (average of 2 sides)	1.2
Mean	2.4

As the sides from the various plants were not all of the same age when received at the laboratory, it would be incorrect to assume that all of the variation in tenderness of product between plants was ascribable to differences in the curing practices employed. Nevertheless, in view of the relation between tenderness and pH previously observed in samples of raw pork adjusted to

various degrees of acidity and alkalinity by injections of lactic acid or ammonia solutions (6), the effect of pH of pickle (3) on the toughness of the present series of samples was investigated. Omitting four plants for which the analytical data were incomplete, statistically significant correlation coefficients of -0.58 between the cutting force required and the product of pH of pump pickle (mean of two samples) $\times$ number of "stitches", and of $+0.48$ between cutting force and the product of pH of cover pickle $\times$ number of days in cure were obtained from the data for the remaining 18. There was no appreciable correlation ($r = -0.09$) between the two acidity indices for the same plant, with the result that the partial correlation of each index with toughness, independent of the other, at -0.61 and $+0.52$ respectively, did not differ significantly from the simple value. It is to be noted that the average pH of the pump pickles, at 7.6, was on the alkaline side of neutrality, and that of the cover pickles, at 6.6, was on the acid side, but in view of the fact that the pH of the meat itself on receipt at the laboratory, although previously shown to be affected to some extent by the pH of the pickles (3), nevertheless varied only within narrow limits, and could not be demonstrably related to toughness on the number of observations available, the mode of action of the pickles in modifying this last property remains obscure.

Coefficients of correlation between toughness and salt, moisture and nitrate content (2), as well as number of days from curing to receipt at the laboratory, were also computed, but failed to indicate any significant relation.

Effect of Heat Treatment on Tenderness

The heat treatments represented in Series (ii) comprised six temperatures, namely, 20, 40, 50, 60, 70, and 80° C., and four periods of heating, 5, 10, 20, and 40 hr. The experimental procedure, which was designed primarily to study the effect of heat treatment on nitrite content and colour, and has been described in detail elsewhere (5), may be summarized as follows: Sixteen "backs" cut from individual sides were allotted at random, four to each of the four periods of heating, and a piece from each back was allocated, also by a random process, to the six temperatures maintained for each period. The individual pieces, having been trimmed free of the cut portions of the ribs and the layer of back fat, were wrapped first in lightly waxed glassine paper and then in kraft paper, and heated in ovens maintained at the specified temperatures. At the conclusion of heating, the surface layers of each piece were trimmed off prior to the selection of suitable samples for the determination of tenderness.

Table II shows the analysis of variance of the measurements on the individual pieces, and Table III summarizes the treatment averages (means of four pieces). Unfortunately, through an oversight, the pieces which should have been heated at 20° C. for 40 hr. were removed at the end of 20 hr.; and those heated at 80° C. for 40 hr. were dehydrated to such an extent that satisfactory measurements of toughness could not be made. The tests of significance in Table II are therefore deduced from the data for 40 to 70°, in

TABLE II

ANALYSIS OF VARIANCE OF TOUGHNESS (RELATIVE WORK DONE IN CUTTING) OF BACON SAMPLES SUBJECTED TO VARIOUS HEAT TREATMENTS (40 TO 70° C.)

Variance due to	Degrees of freedom	Mean square
Period of heating (average for all temps.)	3	0.77
Error *(a)*	12	1.10
Temperature of heating (average for all periods)	3	3.96*
Interaction, temp. × period	9	3.30**
Error *(b)*	36	1.02

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*

which region the factorial combination of treatments was complete. Table III however includes all of the available observations.

Owing to the fact that the pieces cut from the same back resembled each other more closely than did those from different backs, the comparisons afforded by the experiment are of two levels of precision, as indicated by mean square errors *(a)* and *(b)* in Table II. Over the temperature range 40 to 70°, the differences between the average toughness after the four periods of heating did not attain statistical significance on the number of observations available. This however was due to a balancing of effects, rather than to an absence of any influence of duration of heating, as, in addition to average temperature effects, a significant interaction of temperature with period of heating is demonstrated in the lower part of this table.

The maximum average toughness, over all four heating periods, was observed after heating at 50° C., but owing to the interaction noted above the maximum for the individual periods varied from 60° for the 5 and 10 hr. periods to 40°

TABLE III

AVERAGE TOUGHNESS (RELATIVE WORK DONE IN CUTTING) OF BACON SAMPLES SUBJECTED TO VARIOUS HEAT TREATMENTS

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–70°
	20	40	50	60	70	80	
5	3.05	4.05	3.40	4.25	4.28	3.02	3.99
10	3.02	3.75	3.78	4.40	3.02	2.98	3.74
20	3.63	4.35	4.65	3.75	3.48	1.58	4.06
40	—	3.65	5.85	2.78	2.08	—	3.59
Mean	3.31	3.95	4.42	3.79	3.21	2.52	3.84

for 20 hr. and 50° for 40 hr. With the exception of the anomalous value observed after heating at 40° C. for 40 hr., the general tendency was for toughness to increase with duration of heating at temperatures of 20 to 50°, but to decrease with the duration of temperatures of 60 to 80°. It has to be remembered of course that these results were obtained from small pieces of meat, and that the temperature and time effects noted are therefore unlikely to be directly applicable to the commercial treatment of entire sides. Nevertheless, the bearing of these facts upon the operations of commercial smoking will be appreciated, as temperatures ranging from as low as 40° in the smoking of bacon, to 60° or more in the production of "pre-cooked" hams are employed in practice. The reasons, other than possibly the straightforward removal of moisture, for the increase in toughness with time of heating at temperatures up to 50° are at present unknown, but it is of interest to note that the higher temperatures to which "pre-cooked" products are subjected may be expected to have a definitely tenderizing effect.

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